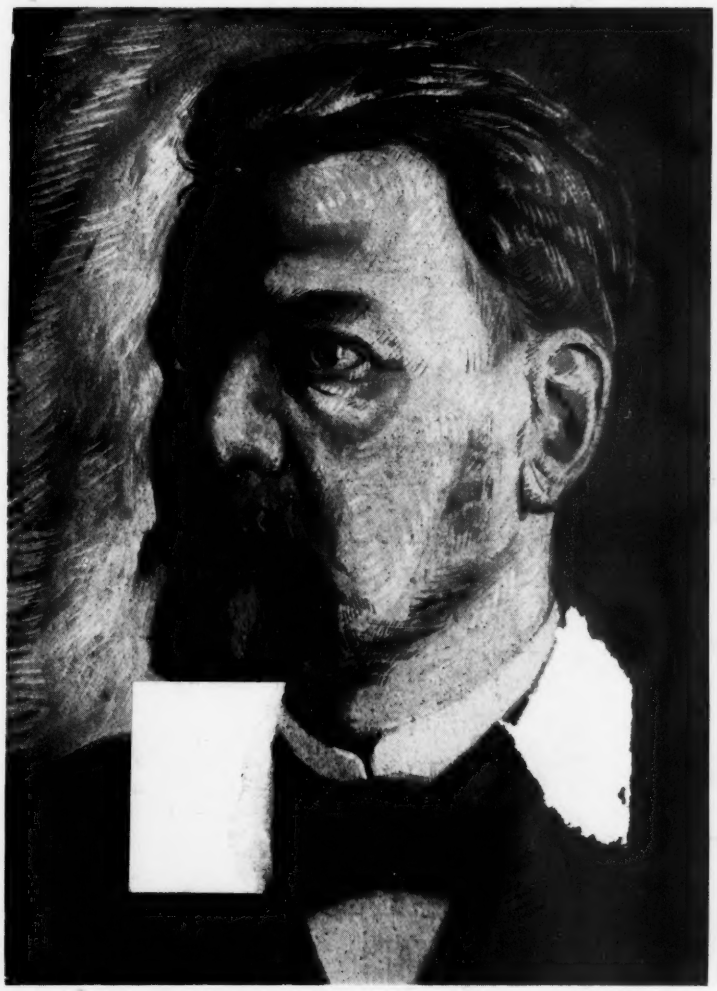
DIABETES

The Journal of the American Diabetes Association



S. G. CHASSOVNIKOV

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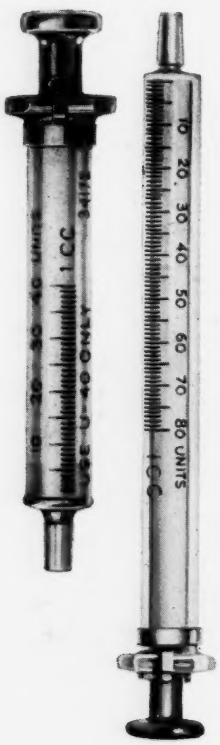

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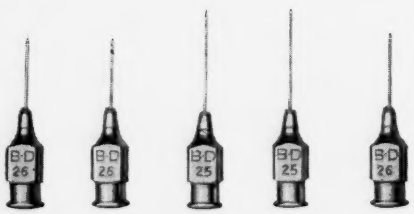

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The Banting Memorial Lecture 1958

The Prediabetic State in Man

Definition, Interpretation and Implications

Jerome W. Conn, M.D., Ann Arbor

It is with pride, humility and a sense of important responsibility that I begin the presentation of a Banting Memorial Lecture.

The problem which I have chosen to discuss today is one with which my group has been concerned during the past eleven years. It has to do with the effect of ACTH and glucocorticoids upon carbohydrate tolerance of normal people. Before proceeding further I wish to point out that today I act as spokesman for the various past and present members of my research staff who have contributed their ideas and efforts to this long-term project. The list of names begins back in 1947 with Dr. Margaret Johnston, Dr. Lawrence Louis and Dr. Clayton Wheeler and concludes with that of my present associate Dr. Stefan Fajans who, for the past five years, has carried the major responsibility for this project. My own function throughout this effort has been that of advisor in experimental design and in interpretation of data. Financial support for a major portion of this eleven-year period has come from the United States Public Health Service.

I do not wish to dwell on the historical aspects of the influence of adrenal cortical function upon carbohydrate metabolism. However, I would like to indicate some of the landmarks for purposes of orientation. Clinicians have been aware of the occurrence of spontaneous hypoglycemia in patients with Addison's disease since 1909 when this phenomenon was first described by Porges.[1] Following the description by Harvey Cushing of the syndrome which bears his name it was soon evident that a large percentage of such patients are frankly diabetic,[2] and that almost all of the rest can be shown to have decreased carbohydrate tolerance when glucose-loading tests are employed.[3] In 1935 Long and Lukens[4] established the ameliorating effect upon pancreatic diabetes of bilateral adrenalectomy in the cat. Long, Katzin, and Fry,[5] in a beautifully designed study published in 1940, demonstrated, among many other things, that the partially depancreatized rat was much more susceptible to the diabetogenic effect of adrenal cortical extracts than were animals with pancreas intact. Ingle and his associates[6,7] produced hyperglycemia and glycosuria in intact force-fed rats by administration of large amounts of either adrenal cortical steroids or ACTH.

In 1947 purified preparations of ACTH became available in sufficiently large quantities for testing in man. There was, of course, still no hint that ACTH would be of any value as a medicine, except possibly in hypopituitarism, and Kendall had not yet synthesized cortisone. Our own studies with ACTH began at that time and were designed to determine the over-all metabolic effects of this substance in normal men and women. At the 1948 meeting of this Society we reported[8] the production of temporary diabetes in normal people by the administration of ACTH for a five- to ten-day period. Table 1 shows some of the data from the very first subject so studied. It will be noted that upon administration of ACTH there began a significant glycosuria, elevation of the fasting blood sugar, a great increase in urinary nitrogen, and a severe loss of tolerance for carbohydrate. All of these changes reverted quickly to normal when ACTH was stopped. These experiments were replicas of those which had been done in rats two years before[6] but they demonstrated for the first time that in man, too, a state of continuous hyperglycemia and glycosuria could be induced by the administration of large doses of ACTH.

By the 1949 meeting of this society, Kendall had already synthesized cortisone, and Sprague, Mason, and Power[9] reported the production of diabetic glucose tolerance curves in normal people given 200 mg. of cortisone daily. They, too, observed the rapid return of normal carbohydrate tolerance upon cessation of cortisone administration.

In 1950 we[10] gave in a paragraph the basis for what

Presented at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco on June 21, 1958.

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TABLE 1

Effect of ACTH upon carbohydrate and nitrogen metabolism
(R.S.—normal man—thirty-seven years old)

	Days	F	Blood Sugar* mg./100 cc. ½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	Urinary Sugar* gm./ 24 hr.	Nitrog. gm./ 24 hr.
Baseline Period	1	83						—	—
	2	85						1.3	12.0
	3	93						1.1	13.5
	4	85						1.2	13.6
	5	87						1.3	14.4
	6	82	134	100	92	100	49	1.0	15.5
ACTH 105 mg./day	1	77						13.8	15.8
	2	115						16.0	18.4
	3	120						21.0	18.8
	4	121						22.0	20.9
	5	130	227	226	241	201	88	38.4	21.6
	6	124						33.0	22.3
	7	124						31.0	21.9
	8	127						36.0	23.2
Recovery Period	1	125	180	182	235	145	51	12.0	18.5
	2	66						1.4	18.2
	3	66						1.2	16.9
	4	63						1.2	15.3
	5	64						1.0	14.4
	6	75						1.0	14.6
	7	81						1.0	15.0
	8	79						1.0	15.0
	9	84						—	—
	13	90	158	86	107	81	62	*Somogyi	

has evolved as the cortisone-glucose tolerance test for detecting susceptibility to diabetes. I quote this paragraph because it represents the starting point with respect to much of the newer data that I shall report today. "We have observed that the individual who is susceptible to the diabetogenic effect of ACTH, is susceptible as well to the diabetogenic effect of cortisone. On the other hand, individuals *resistant* to the diabetogenic effect of ACTH are also resistant to the same dose of cortisone which produces diabetes in the ACTH-susceptible person. These findings indicate that a major difference between *diabetes susceptibility* and *diabetes resistance* among so-called normal people given ACTH, lies in end-organ responses to '11-oxysteroid' activity rather than to qualitative or quantitative differences in adrenal steroid production." In the same year Wilson, Frawley, Forsham, and Thorn[11] reported that a functional relationship appeared to exist between the pancreatic islets and the adrenal cortex in man. They concluded that loss of carbohydrate tolerance upon the administration of ACTH (or resistance to loss of carbohydrate tolerance) might well be an indication of the reserve function of the beta cells.

In the course of our own studies we had made two additional observations which seemed to be generally applicable in people with normal carbohydrate tolerance. First, if the dose of cortisone is sufficiently large *all* people respond with an *initial* decrease in carbohydrate tolerance. Depending upon the dose of cortisone employed, the decrease of carbohydrate tolerance may reach its peak of intensity in four or five days. In the great majority of normal people, tolerance begins to be regained thereafter despite continuation of the dose of cortisone. We interpret this as an attempt on the part of the beta cells to compensate for (1), an increased peripheral resistance to insulin activity and (2), the increased glyconeogenesis induced by the administered cortisone.

The second observation (a very obvious one) was that as the dose of cortisone was reduced the early decrease in carbohydrate tolerance of normal people (before compensatory mechanisms are evident) becomes less and less. We then had two ends of a spectrum. At one end the dose of cortisone could be made sufficiently large that it would diminish carbohydrate tolerance of everyone. At the other end no detectable effect on carbohydrate tolerance of normal people could be observed if the dose were made sufficiently small.

Since we had found that among *apparently* normal individuals marked differences exist in susceptibility to, or resistance to, loss of tolerance produced by administration of fairly *large* doses of cortisone, we wondered if we could find the critical single dose of cortisone which would not effect, significantly, the carbohydrate tolerance of normal people but which might, on the very first day of administration and before pancreatic compensations had had a chance to occur, disclose people susceptible to future diabetes. This, of course, assumed that all of the people so tested would have perfectly normal baseline, standard glucose tolerance tests.

The obvious place to look for people who might be susceptible to diabetes is in the families of known diabetics. The control group had to be people who could not recall ever having heard of a diabetic in their families. Most of you are familiar with our first report in 1954[12] on this long-term project and of the short subsequent follow-up report in 1955.[13] Since then we have enlarged the various groups under study and have been able to do follow-up testing in some of the subjects from one to four years after they had been classified initially as positive or negative reactors to the cortisone-glucose tolerance test.

A few words are needed about our methods and criteria. Throughout these studies the Somogyi-Nelson method has been used for the determination of sugar of venous blood. Glucose tolerance tests have all been oral, 1.75 gm. of glucose per kilogram of ideal body weight having been used as the glucose load. Each glucose tolerance test has been preceded for at least three days by a diet maintenance in calories, and containing 300 gm. of carbohydrate per day. With respect to the glucose tolerance test preparatory diet I might say parenthetically that we are well aware that normal carbohydrate tolerance can be preserved at carbohydrate intake levels which are far below 300 gm. per day. We think, however, that the diet which precedes a glucose tolerance test (a) must have a sufficiently safe plethora of carbohydrate that it is capable of awakening the dormant beta cells of anyone who may have been restricted in dietary carbohydrate for weeks or months and (b) that *whatever* the plethoric level which may be decided upon, it should be *standard* for all adults.

In the 512 subjects who provide the data for the present report, the dose of cortisone employed in the cortisone-glucose tolerance test was as follows: If the subject weighed less than 160 lb. he was given orally 50 mg. of cortisone acetate eight and one half hours and again two hours before a standard glucose tolerance test was performed. If total body weight exceeded 160 lb. the dose was 62.5 mg. orally at both of these same times before the glucose tolerance test.

When one attempts to classify large numbers of individuals on the basis of a standard glucose tolerance test and a standard cortisone-glucose tolerance test he is obliged, initially at least, to set up somewhat arbitrary lines of demarcation. He must then stick rigidly to these criteria until enough information has been collected to indicate that a change in any individual criterion is required. The present study will eventually provide such information. It can be obtained in no other way. For example, what, under conditions of standard dietary preparation is the smallest decrease in carbohydrate tolerance which can be confidently diagnosed as indicative of *presence* of diabetes mellitus? A long-term study of this nature in a large number of individuals has never been carried out. In any case, for purposes of classification and with the methods described above, we regard as indicative of diabetes a glucose tolerance test which gives a value of 160 mg. per cent or higher in the first hour, 140 mg. per cent or higher at one and one half hours, 120 mg. per cent or higher at two hours, figure 1. A curve which is below 160 at one hour and below 110 at two hours is regarded as indicative of normal tolerance. When the two-hour value falls between 110 and 120 mg. per cent we classify it as belonging to a special group which we designate as *probable diabetes.*

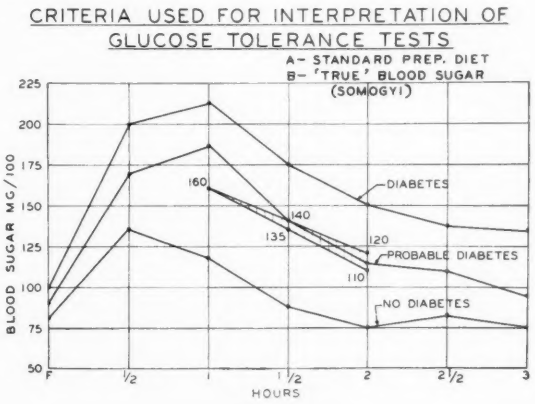

FIGURE 1

The criteria which I have indicated as being diagnostic of diabetes are in close agreement with those careful studies of this problem reported by Mosenthal and Barry,[14] and by Moyer and Womack.[15] Our own experience over the past twenty-five years indicates that the 160, 140, 120 criterion (figure 1) has never missed. I wish to show just a few examples.

Table 2 shows the importance both of a one-hour value over 160 and of a two-hour value over 120. Eleven years later the curve was obviously diabetic.

TABLE 2

L. H., male, height 6′ 2″

Year	Age	Weight	Glucose Tolerance Test F	1 hr.	2 hr.	3 hr.
1939	59	240	83	186	122	58
1950	70	227	134	262	304	205

Table 3 shows the importance of the two-hour value of 120 or above. Even by our own criteria, the one-hour value should have been 160 or above for a diagnosis of diabetes. Nevertheless, one month later islet cell decompensation has occurred and the diagnosis is evident. The third curve is one obtained for comparative purposes seven years later.

TABLE 3

G. B., male, height 5′ 5″

Year	Age	Weight	Glucose Tolerance Test F	1 hr.	2 hr.	3 hr.
April 1934	54	162	77	151	120	84
May 1934	54	162	150	284	230	121
1941	61		220	364	348	302

Table 4 demonstrates the case of a six-month old child with furunculosis for six weeks and glycosuria for two weeks. The first-hour value of the glucose tolerance test is not quite 160 but the second-hour value is significantly delayed. The furunculosis and glycosuria then disappeared and the boy was well until the age of fourteen when within one week he progressed from polyuria and polydipsia to diabetic coma. If at the age of twelve someone had noted capillary microaneurysms in his fundi, this case could have been used erroneously to illustrate the appearance of vascular lesions before any defect in carbohydrate metabolism had been known.

TABLE 4

F. P., Male 1938—*Age 6 months*

Furunculosis for six weeks
Glycosuria for two weeks
Glucose tolerance test

F	½	1	2	3
84	172	153	129	105

1951—*Age 14 years*

Well and asymptomatic until:
12/2 Sore throat, anorexia
12/6 Polyuria and polydipsia
12/7 Air hunger, confusion
12/8 Coma, FBS: 506 mg. per cent
CO_2 comb. power: 5 mEq./L.
NPN: 92 mg. per cent

Table 5 represents the case of a thirty-nine-year-old woman whom we diagnosed as having renal glycosuria in 1938. The test was repeated three years later and one notes that while the first-hour value never went above 153, the second-hour value of 130 made this curve extremely significant. Eleven years later diabetes is obvious.

TABLE 5

R. K., female, height 5′ 3″

Year	Age	Weight	Glucose Tolerance Test F	1 hr.	2 hr.	3 hr.	4 hr.
1938	39	128	82 0	112 ++	95 +++	85 +	64 0
1941	42	128	101 0	153 +++	130 ++++	92 ++++	93 +++
1942	43	127	162 ++++	284 ++++	318 ++++	260 ++++	146 ++++

We mentioned that when the two-hour value falls between 110 and 120 we designate this as probable diabetes. Table 6 represents a case in point. A one-hour value of 170 and a two-hour value of 115 were obtained in 1934. Two years later the one-hour value was about the same but the two-hour value indicated a further delay in the patient's capacity to clear the blood of a glucose load. Twenty years later, still with a normal fasting blood sugar, the diagnosis of diabetes mellitus is clear.

TABLE 6

A. W., male, height 5′ 7″

Year	Age	Weight	Glucose Tolerance Test F	1 hr.	2 hr.	3 hr.
1934	46	149	81	170	115	89
1936	48	149	86	166	150	100
1956	68	150	84	201	241	151

The last example (table 7) tells an interesting story. On March 18, 1938, the diagnosis of diabetes mellitus was clear. Eleven days later, with no treatment of any kind, pancreatic compensation has occurred and the only thing abnormal is a slight two-hour delay with a level of 112 indicating probable diabetes. In 1939 and 1941 glycosuria was found but nothing was done about it. Thus, from 1938 to 1946 the patient was untreated and was asymptomatic. In 1946 he had furunculosis with polyuria and polydipsia and an eight-pound weight loss. He again recompensated without treatment. One year later he developed an abscess of the abdominal wall, his fasting blood sugar was found to be 350 mg. per cent, and treatment with insulin was begun. A year later there was retinitis proliferans. When one speaks of diabetic

TABLE 7

B. S., male, height 5′ 6″, weight 152 pounds

Date	Age	Glucose Tolerance Test F	1 hr.	2 hr.	3 hr.
3/18/38	24	214	375	353	273
3/29/38	24	107	146	112	99
1939	25	Asymptomatic glycosuria on insurance exam.			
1941	27	Asymptomatic glycosuria on draft exam.			
1946	32	Furunculosis, polydipsia, polyphagia, 8 lb. loss of weight. Spontaneous recovery without treatment.			
1947	33	Abscess of abdominal wall. FBS—350 mg. per cent. Rx—Insulin and diet.			
1948	34	Retinitis proliferans, vitreous hemorrhages.			

retinopathy in the presence of normal glucose tolerance it will be well to remember the curve in this series which is marked by the date of March 29, 1938. I think that this illustrates, too, that the old definition of diabetes mellitus, a state of continuous hyperglycemia and glycosuria, is not only antiquated but dead despite the fact that many of our present-day clinical experts still embrace it.

Thus, it should be clear that as our knowledge of the life history of diabetes mellitus increases we should be able to recognize its existence at a progressively earlier time. In any case, using the *arbitrary* criteria which I have outlined as separating diabetic patients from nondiabetic individuals, we have been able to make a number of interesting observations.

Figure 2 shows the results of the standard baseline glucose tolerance test (not the cortisone-glucose tolerance test but the ordinary glucose tolerance test) when applied, on the one hand, to 387 close relatives of diabetic patients and, on the other, to 125 people who know of no diabetes in their families. Note that among the apparently healthy relatives of known diabetics 18 per cent are already diabetic without knowing it.

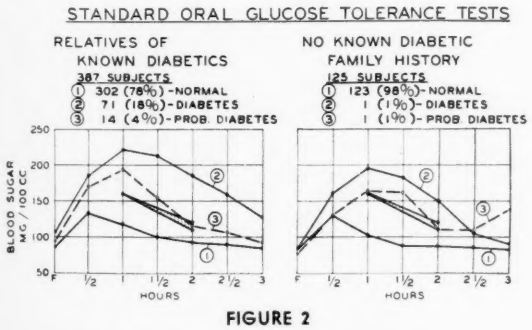

FIGURE 2

Another 4 per cent are *probably diabetic* and we shall have more to say about this group later. Thus, somewhere between 18 and 22 per cent of close relatives of diabetics are themselves already diabetic. Of 125 subjects with no *known* family history of diabetes only one subject showed a diabetic glucose tolerance test and only one more had a curve indicating that he was probably diabetic. This figure of less than 2 per cent in the control group stands in marked contrast with the 22 per cent figure found among relatives of known diabetics.

Thus, before ever getting to the *cortisone-glucose tolerance* test, very significant conclusions can be drawn from the standard glucose tolerance test. If one wishes to detect the unknown and undiagnosed diabetics in this country the area is all marked out for him. Mass screening programs of the general population seem a bit cumbersome when one already knows that he can obtain an 18 to 22 per cent positive yield by concentrating initially on a well delineated population group. The fact is that if local, urban, state or even national registries were set up for diabetic patients one could, within just a few years, detect essentially all of the undiagnosed diabetics in this country. This is true because if all of the close relatives of known diabetics were to be screened this year we would detect the vast majority of unknown diabetics. A similar screening procedure during succeeding years would give rapidly decreasing percentage yields until we arrived at a figure representing new cases which had developed during that particular year.

Before leaving figure 2 let me outline what we have done with these various groups. Obviously, the group of seventy-one patients who already have diabetes do not require a cortisone-glucose tolerance test. They are, therefore, eliminated so far as the studies for *diabetes susceptibility* are concerned. Our major interest from now on then is concerned with the 302 subjects who have perfectly normal glucose tolerance tests, and with the 123 control subjects who have an almost identical baseline glucose tolerance test as that exhibited by the nondiabetic segment of the relatives of known diabetics.

The cortisone-glucose tolerance test is applied to both of these nondiabetic groups as a possible measure of *susceptibility* to diabetes.

Figure 3 shows the results of the cortisone-glucose tolerance test in nondiabetic relatives of known diabetics. There are 259 subjects in the group, every one of whom has a perfectly normal baseline glucose tolerance test. Before interpreting these results I wish to emphasize two points regarding criteria. First, I am sure that regardless of what I believe there are some who will not agree that the 160, 140, 120 line (the upper line of

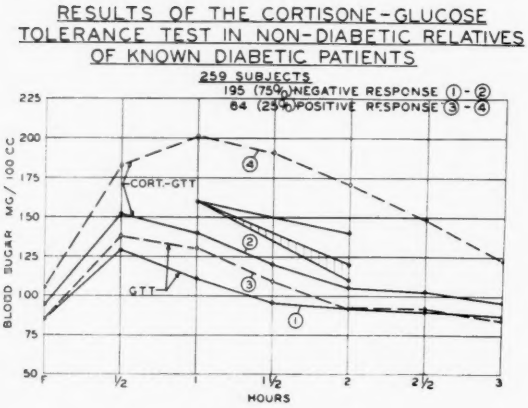

FIGURE 3

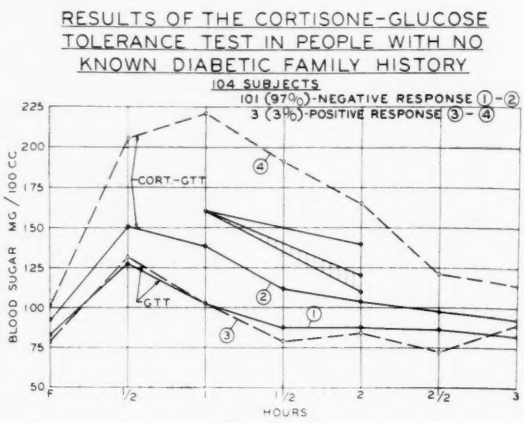

FIGURE 4

the shaded area) is sufficiently high to be certain that diabetes mellitus exists. On the other hand, I think there would be unanimity of opinion that a curve falling *below* the lower line of this area, namely, 160, 135, 110, would certainly be indicative of the *absence* of a defect in carbohydrate metabolism. Thus, we shall now be dealing with a group of people which everyone will agree is not diabetic. Secondly, you will note that a new higher line has been added. This line runs from 160 at one hour to 140 at two hours. This is the line of demarcation which initially we set arbitrarily as separating a positive cortisone-glucose tolerance test from a negative one. Thus, in order for an individual to react positively to this test, his baseline glucose tolerance test must fall below 160 mg. per cent at one hour and 110 mg. per cent at two hours and his *cortisone-glucose tolerance test* must be above 160 at one hour and above 140 at two hours.

It will be seen that 75 per cent of the subjects gave a negative response (lines 1 and 2), while sixty-four individuals, or 25 per cent, (lines 3 and 4) gave a positive response.

When one now compares the figure of 25 per cent positive responders among nondiabetic relatives of diabetics, with what is found in the control group he becomes confident that this test is making some type of important distinction among nondiabetic relatives of diabetic patients. In figure 4 it will be observed that although the baseline glucose tolerance tests are identical, 97 per cent of the control subjects give a negative response. Thus, less than 3 per cent of the controls react as 25 per cent do when there is a family history of diabetes. This figure of less than 3 per cent for the control group may drop to less than 2 per cent since it now appears that one of the three subjects who gave a positive response may have found a diabetic patient in her family.

Now let us (figure 5) see what the cortisone-glucose tolerance test says about the fourteen people whose *baseline* glucose tolerance tests fall into the group which we have classified as *probable diabetes*. This, you will recall, was defined as the curve in which the two-hour blood sugar value falls between 110 and 120 mg. per cent. Of the fourteen individuals, twelve (86 per cent) gave a positive cortisone-glucose tolerance test (curves 3 and 4). Curve 1 is that of the other two subjects on the baseline test and curve 2 is their cortisone-glucose tolerance test. It will be observed that even these two individuals had *almost* a positive cortisone-glucose tolerance test. Obviously, only the future holds the answers that we

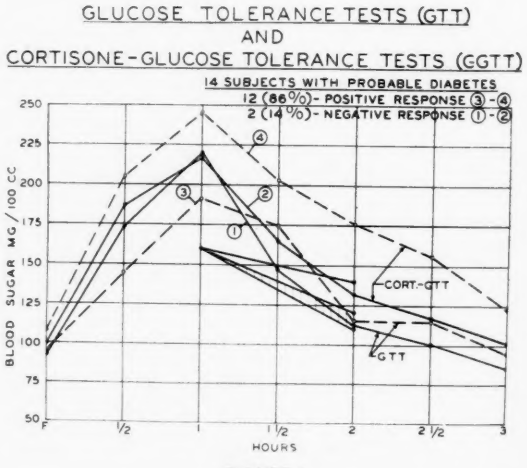

FIGURE 5

would like to know here but it is very significant that 86 per cent of those that gave a borderline baseline test were positive on the cortisone-glucose tolerance test. This figure (86 per cent) is to be compared with the figure of 25 per cent positive cortisone-glucose tolerance tests when the baseline curve was *normal* and there was a family history of diabetes.

We have evaluated the cortisone-glucose tolerance test in another very interesting group of patients. Table 8 was published nineteen years ago.[16] Some of the more mature members of this audience will recall a group of publications by Newburgh and me between 1937 and 1940 which dealt with the effect of weight loss and weight gain upon carbohydrate tolerance of obese, middle-aged diabetics. The data on table 8 exemplify what we observed many times. One notes that a diabetic curve reverts to normal with a return of the weight to normal. As weight is regained the test becomes typically diabetic again and again reverts to normal with the attainment of a normal weight. Inasmuch as we *know* that these people are diabetic to begin with, we thought it would be of interest to see what the cortisone-glucose tolerance test says about them after weight reduction has brought back normal tolerance for carbohydrate by the standard test. Figure 6 shows the results of this experiment upon nineteen subjects whose diabetic glucose tolerance curves reverted to normal after loss of weight. The middle curve is the one obtained before weight loss and is, of course, typically diabetic. The lower one is that which was attained after weight loss and the upper one is the cortisone-glucose tolerance test done at the same lower weight and just a day or two after the standard glucose tolerance test was done. It is to be noted that sixteen of the nineteen individuals, or 84 per cent, gave a positive response. More interesting than that is the fact that three of these subjects *failed* to give a positive response. On figure 7 are shown the data on the sixteen positive responders and on the three negative responders. The results in the latter group may mean that they had attained sufficient return of reserve islet cell function that they were able to resist the stress placed upon the islets by the cortisone-glucose tolerance test, *or* it could mean that a different mechanism is responsible for the original loss of carbohydrate tolerance in this subgroup. It could also mean that on the basis of the criteria as they are now set up we can expect no more than about 84 per cent accuracy in picking out the potentially diabetic individual. But even 84 per cent would be acceptable temporarily. At this point it would be well to remember another figure (86 per cent) that I emphasized earlier. This was a figure of 86 per cent positive reactors among people whose baseline curves we classified as *"probable diabetes."*

TABLE 8

Effect of obesity on glucose tolerance
(J. D., male, forty-eight years old)

Overweight Per cent	Glucose Tolerance Test F	1 hr.	2 hr.	3 hr.
45	128	314	322	202
0	91	140	72	61
25	119	230	185	102
0	87	135	107	66

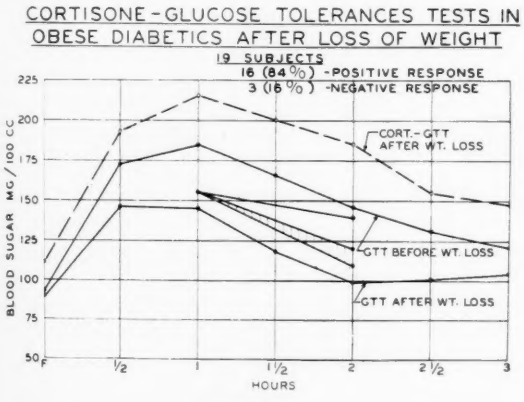

FIGURE 6

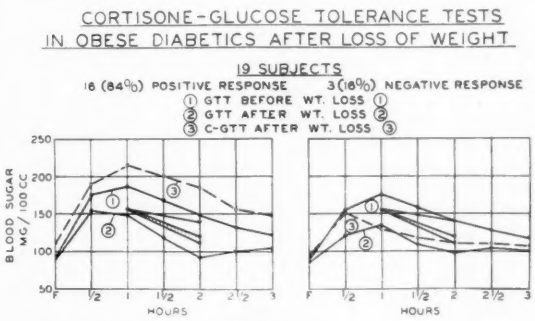

FIGURE 7

Table 9 shows another aspect of the problem of obesity, diabetes and loss of weight. This very obese female was diabetic by our criteria in October, 1952. During the next six months she lost twenty-two pounds and compensated sufficiently to exhibit a normal standard glucose tolerance test. The cortisone-glucose tolerance test, however, was positive. One year later, at the same weight, the *standard* glucose tolerance test was clearly diabetic and the cortisone-glucose tolerance test was positive. This is of particular interest since it shows

TABLE 9

Obesity, diabetes, loss of weight and the cortisone-gtt (Female, 5' 1½")

Time	Age	Wt.	F.	½	1	1½	2	2½	3
Oct. 1952	47	154	99	204	198	154	120	74	65
Mar. 1953	48	132	93	157	167	155	86	86	90
Cortisone-GTT			129	230	284	258	213	184	165
Mar. 1954	49	134	92	169	238	228	138	97	54
Cortisone-GTT			127	255	306	297	278	184	133

that return of normal carbohydrate tolerance after weight loss as measured by the *standard* glucose tolerance test in an obese middle-aged glycosuric patient, does not mean that progressive loss of tolerance will not occur with time even *without* gain of weight. In fact, this has been the result in many of the cases that we reported twenty years ago. The other point of interest here is that had this patient been seen first in March of 1953, the cortisone-glucose tolerance test would have indicated correctly that she would eventually become diabetic.

Figure 8 shows a typical example of individual subjects who have gone on to develop diabetes mellitus as the cortisone-glucose tolerance test had suggested that they might. It demonstrates the normal baseline glucose tolerance test and the positive cortisone-glucose tolerance test in September, 1956; and in October, 1957 undoubted diabetes mellitus.

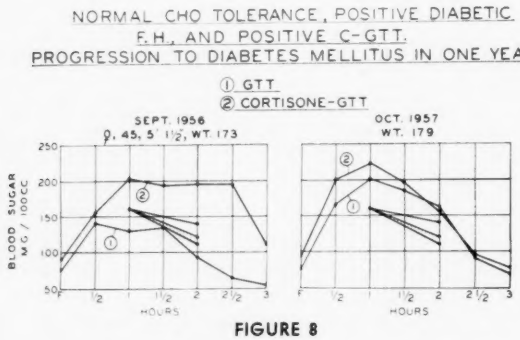

FIGURE 8

Figure 9 emphasizes the probable importance of a one-hour blood sugar level of 170, all other parts of the curve being normal. Most experts in our field would consider the number one curve in 1956 as normal. The cortisone-glucose tolerance test was positive. Two years later the patient was clearly diabetic. Obviously long-term studies of this nature will allow *all* of us to sharpen up our present criteria for the presence and absence of diabetes mellitus and to discard outmoded ones.

We have been able, to date, to do follow-up glucose

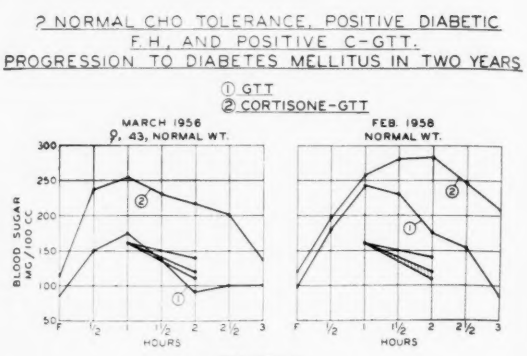

FIGURE 9

tolerance tests and follow-up cortisone-glucose tolerance tests in seventy-one subjects who were related to diabetics, but whose standard glucose tolerance tests were normal (table 10). Of these seventy-one nondiabetic relatives of diabetics, thirty had shown a positive cortisone-glucose tolerance test, and forty-one a negative one. Up to May 1, 1958, four (13 per cent) of the thirty with positive cortisone-glucose tolerance tests have developed diabetes and three more (10 per cent) have developed the curve which we classify as probably diabetic. Of forty-one negative reactors *with* a family history of diabetes, only one person (2 per cent) has developed an abnormal curve. Thus, of thirty positive reactors, 23 per cent have developed diabetes or *probable diabetes* while only 2 per cent of the negative reactors are now diabetic. Of the control group (no family history of diabetes and a normal glucose tolerance test) *none* so far retested has developed an abnormal curve. Thus, even after five years with this type of study, while the direction of the results seems clear, the numbers are still too small to be certain of their meaning. But persistent follow-up of these various groups and subgroups over the *next* five years will give much more information than has been obtainable to date because of the very nature of the study.

Finally, we have observed a very small group of positive reactors and a very small group of negative reactors (both with family histories of diabetes) which

TABLE 10

Over-all follow-up results in the group of nondiabetic relatives of diabetics (1 to 4 years)

Cortisone-GTT	Number Subjects	Now Diabetic		Probably Diabetic	
		Number	Per cent	Number	Per cent
Positive	30	4	13	3	10
Negative	41	1	2	0	0

have, upon repeated testing with the cortisone-glucose tolerance test, varied back and forth between positive and negative responses. This type of instability of carbohydrate metabolism has not been observed *at all* in any of the individuals without a family history of diabetes.

However, the numbers in these groups are too small to lend significance to any conclusions about this phenomenon.

DISCUSSION

Since the beginning of this project, back in 1950, we have had one major objective. It has *not* been to devise a test which would measure the reserve functional capacity of the pancreatic beta cells. It has *not* been to be able to say that Johnny *will* and Mary *won't* develop diabetes. Our goal has been to find Johnny and to study *him* and many like him. The major objective has been to pinpoint the potential diabetic before the disease is evident by present testing methods in order to study what processes exist to make this particular individual vulnerable to various factors which precipitate the clinical syndrome. Clearly, the ultimate goal of all studies which concern themselves with the etiology of disease is prevention or cure of that disease.

Our own purposes and goals, therefore, have defined for us the so-called "prediabetic state." In an individual destined to become diabetic, the prediabetic state must be regarded as existing from the time of conception to the time that a definitive diagnosis can be made by present methods of testing. Thus, defined, the prediabetic state becomes a target which will continue to move toward the goal of eventual complete understanding. What may be regarded as prediabetic today will later be shown to be a clear manifestation that the disease is *present*. For example, there is now but one way to make a clinical diagnosis of diabetes mellitus with certainty. It is to demonstrate diminished tolerance for carbohydrate under certain standard conditions. Even here there is room for argument as to what does and does not constitute diabetes mellitus. Let us assume, however, that over the next five to ten years the cortisone-glucose tolerance test, or some modification of it, can be shown to predict accurately those individuals who will eventually lose carbohydrate tolerance and become diabetic by *present* criteria. Would we then not be justified in changing our criteria for the diagnosis by instituting the cortisone-glucose tolerance test as the more sensitive indicator of decreased carbohydrate tolerance? We would then be making the diagnosis of diabetes at a much earlier time in the lives of these individuals and perhaps we could learn to do something of value for them during this period which we now call latent. The *prediabetic* state would then be changed to that period of time which exists before the *cortisone-glucose tolerance test* becomes positive. What I wish to say is that the prediabetic state must be defined in terms of both time and objective manifestations, each newly discovered manifestation taking us further back into the prediabetic state. At the present time the index which takes us back the furthest into the prediabetic state is the remarkable correlation between very large babies at birth and the very high incidence of future diabetes in the mothers of these children. But other indices which can be applied to our enormous prediabetic population must be found.

I think that one can say now without question that there is an hereditary aspect involved in diabetes mellitus. Thus, the prediabetic state exists from the moment that the ovum is fertilized to the time that we can justify a clinical diagnosis by *whatever* means. Hoet,[17,18] Jackson[19,20] and others have emphasized the probable importance of the in utero environment upon the fetus. It has been suggested that this *congenital aspect* (the as yet unknown abnormality contained in the prediabetic mother which influences the fetus) may have even greater importance than the hereditary one itself. I think it is best, in the light of present knowledge, to regard the hereditary aspect as basic and to consider that from the time of conception to death there occur many influences which can bring to the clinical horizon evidence that the diabetic syndrome exists. Among the poorly understood influences which may bear importantly upon the future status of the hereditarily determined prediabetic individual are (1) the nature of the in utero environment of the prediabetic mother which produces large babies and/or hyperplastic islets of Langerhans[21] and (2) the modi operandi of the various stressful situations known to be associated with decreases of carbohydrate tolerance in prediabetics, i.e., infection, puberty, pregnancy and obesity.

A major first step must consist of an ability to find the so-called prediabetic individual. While perfectly true, it is grossly insufficient to point out that a prediabetic female can often be spotted thirty years in advance of her loss of carbohydrate tolerance by the fact that she has delivered a ten and one-half pound baby. Similarly, the fact that the islets of Langerhans of many infants born of prediabetic mothers are hyperplastic[21] (as indicated by the stillborns of this group) simply gives further evidence that important influences are at work for many years before tolerance for carbohydrate diminishes by

our present methods of testing. But do we not already recognize this to be a fact if we accept heredity to be a basic influence? We know, of course, that the defect in the metabolism of carbohydrate may begin sixty years after birth or only three months after birth.

Finally, I should like to make it clear that in presenting these data to you today we have not implied that glucocorticoids are important in the *etiology* of diabetes mellitus. The cortisone-glucose tolerance test has been devised and is being used as a technical instrument of detection. When used in a standard way it seems to separate the nondiabetic relatives of diabetics into groups which are distinctly different from the groups which are found when the same test is applied to people with no known family history of diabetes. Only long-term future observations on these various groups will tell us whether or not this test, or some modification of it, will be capable of detecting at an earlier age the great masses of people who are to make up our future diabetic populations. When we can detect the prediabetic with reasonable certainty, only then can we justify the use of therapeutic or prophylactic measures designed to prevent the disease or, at least, to prevent the "decreased-insulin-activity-aspect" of the disease, that aspect about which we know the most. We believe that the present study represents a move, *minute* as it may be, in the right direction.

SUMMARIO IN INTERLINGUA

Le Stato Pre-Diabetic In Humanos: Definition, Interpretation, E Signification Ulterior

Le autor delinea le historia, le stato presente, e le possibile promissas futur del test del tolerantia de glucosa post pre-tractamento con cortisona como medio de detection de individuos predisponite a disveloppar diabete mellite.

Le test ha su base in le observation del effecto diabetogene de extractos adrenocortical e—specificamente—in le constatation per le autor e su collaboratores que apparentemente normal individuos exhibi marcate differentias in le grado del depression temporari que es effectuate per satis grande doses de cortisona in lor tolerantia pro glucosa. Le these que iste differentias pote esser utilisate pro differentiar inter individuos pre-diabetic e individuos genuinmente non-diabetic es sub investigation in plure studios, le plus importante del quales esseva initiate in 1954.

In illo, 387 consanguineos de patientes cognoscitemente diabetic e 125 subjectos sin ulle cognoscite historia familial de diabete esseva testate (1) per le test standard de tolerantia pro glucosa sin pre-tractamento con cortisona, sequite, selectivemente, (2) per le mesme test con ille pre-tractamento.

Le cortisona esseva administrate in duo doses equal, 8½ e 2 horas ante le test a glucosa. In individuos con pesos de minus que 160 libras (= 72 kg.), le quantitate total de cortisona esseva 100 mg; in individuos plus pesante, illo esseva 125 mg.

Le resultante curvas de tolerantia esseva interpretate como normal quando le valores del sucro de sanguine esseva minus que 160 mg per 100 ml post un hora e minus que 110 post duo horas. Valores de plus que 160 mg post un hora, plus que 140 mg post un hora e medie, e plus que 120 mg post duo horas esseva considerate como indicatori del presentia de diabete. Individuos con valores de inter 110 e 120 mg post duo horas esseva designate como diabeticos probabile.

Le test de tolerantia pro glucosa (sin cortisona) revelava que 71 del 387 consanguineos de diabeticos (i.e. 18 pro cento) habeva diabete illes mesme. In plus, 14 (i.e. 4 pro cento) debeva esser designate como probabilemente diabetic. Le alteres, i.e. 302 individuos, esseva non-diabetic. Le 125 subjectos sin cognoscite historia familial de diabete includeva solmente un in qui le test indicava le presentia de diabete e un altere in qui le presentia de diabete esseva probabile.

Le test de tolerantia pro glucosa post pre-tractamento con cortisona esseva administrate a 259 del non-diabetic consanguineos de diabeticos e al 123 non-diabeticos sin historia familial de diabete. In le prime de iste gruppos, 64 individuos (i.e. 25 pro cento) reageva positivemente; in le secunde, 3 (i.e. 3 pro cento).

Inter le 14 probabile diabeticos (secundo le test sin cortisona), 12 reageva positivemente in le test con cortisona.

Ex le gruppo del consanguineos de diabeticos, 30 con positive e 41 con negative tests de tolerantia post cortisona esseva tenite sub observation usque al tempore del presente reporto. Quatro del positivos ha jam disveloppate diabete; 3 altere positivos ha devenite probabilemente diabetic. Solmente un del negativos ha devenite diabetic.

Un micre gruppo de subjectos—troppo micre pro permitter generalisationes sed troppo interessante pro esser ignorate—ha oscillate inter positivitate e negativitate in tests de tolerantia a cortisona effectuate in series.

Le autor specula que le disveloppamento de un test pro le detection del stato "pre-diabetic" non es un objectivo terminal. Si il esseva possibile predicer definitemente que un certe individuo va devenir diabetic, il

esserea necessari cambiar le definition de diabete de maniera que illo coperi le presentia del morbo in le individuo nunc designate como pre-diabetic. Le objectivo terminal es comprender le etiologia de diabete ab su presentia latente in le ovo novemente fertilisate, via su stadios "pre-diabetic" in feto e vita postnatal, usque a su declaration definitive a non importa qual nivello de etate. Un tal comprension permitterea alora le cerca de mesuras de intervention therapeutic contra le morbo longemente ante que ha illo comenciate disveloppar le symptomas nunc considerate como characteristic de illo.

In su conclusion le autor sublinea emphaticamente que le recercas hic reportate non significa que glucocorticoides pote esser incriminate como factores in le etiologia o historia natural de diabete mellite.

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Antimetabolites and How They Function

In all living things there are a number of chemical substances which are vitally essential to the intricate series of chemical reactions which is called "metabolism." If any one of these vital compounds is absent from the organism, normal processes fail to occur. Thus the absence of a particular vitamin may lead to death of an animal, or the absence of a particular hormone—let us say one of the sex hormones—can stop a vital process. These essential constituents of living things are called "essential metabolites" or, more simply, "metabolites."

During the last two decades it has been found that if we change the chemical structure of one of these metabolites in any one of several defined ways, the molecule which results is not able to substitute for the metabolite in living processes. Instead, this new chemical relative has the ability to call forth in the organism the specific signs of deficiency of the metabolite to which it is related. Such antagonistic relatives of the metabolites are called "antimetabolites."

The introduction of the antimetabolite concept into pharmacology seems to be causing such a change in thinking about the mode of action of drugs that a revolution may be said to be in progress. Perhaps one of the most significant aspects of this revolution is that reliance on chance for the discovery of the first member of a new series of useful agents is being broken. It is now becoming possible to predict the chemical structure of the first member of a new series and to see these predictions come true.

By D. W. Woolley, from *Perspectives in Biology and Medicine,* Vol. 1, No. 2, pages 176 and 196, Winter 1958, University of Chicago Press.

Action of Insulin and Tolbutamide on Blood Glucose Entry and Removal

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Although it is generally recognized that the blood sugar of the fasting animal is maintained constant by a precise balance between entry and removal rates, it is only recently, with the advent of C^{14}-labeled glucose, that reliable estimations of the rates of these processes have become possible. The availability of tracer methods has also greatly facilitated studies of the mechanism of action of agents which affect the blood sugar level. Such studies, by Searle and Chaikoff,[1] Henderson et al.,[2] Wall et al.,[3] Berson et al.,[4] and Dunn et al.[5] have shed considerable light on the relationships between blood glucose level and its entry and removal rates, and the effects thereon of various hormones. In our own studies,[5] we have been struck by the marked and immediate action of insulin in suppressing the entry of new glucose into the blood of the fasting, normal dog. As a further step in the investigation of this presumably hepatic action of insulin, its effect on the normal and diabetic human has been studied, and its action on the entry and removal rates of blood glucose has been compared with that of the hypoglycemic drug, tolbutamide (Orinase).* These studies are described in the present report.

Though the methods used by different investigators differ in experimental details, they all are similar in principle. Briefly summarized, our method involves the intravenous injection of a "trace" dose of glucose-C^{14} to label the blood glucose. Samples of blood are then removed at intervals for estimation of blood glucose levels and specific activity. The changes in specific activity of the blood glucose, determined by a method involving the specific oxidation of glucose carbon to formic acid, allow calculation of entry and removal rates. Our experimental procedure, and the principles and calculations involved, have been described in our previous study with dogs.[5]

The subjects used in the present study were thirty middle-aged and elderly patients of a chronic disease home and clinic of a large community hospital.* Those classified as nondiabetic had no obvious endocrine disorder, and had a normal two-hour postprandial blood sugar concentration. The thirteen diabetic patients were non- or mildly obese, and were responsive to insulin and tolbutamide. All of the subjects were fasted for at least fourteen hours prior to an experiment, and the diabetics had not received insulin for forty-eight hours. During the course of the experiments they were comfortably at rest in a bed or chair; no food or medication was given and care was taken to avoid any apprehension.

The uniformly C^{14}-labeled glucose (glucose-U-C^{14}) was purchased from the Volk Radiochemical Company. An amount equivalent to 100 microcuries was dissolved in 10 ml. of sterile water and was injected into an arm vein. Samples of blood were removed from a vein in the other arm at regular, frequent intervals. A No. 20 spinal needle ground to a length of 1.5 inches, carrying a stylet moistened with a heparin solution was used. It was possible to keep the needle in the vein throughout the experiment without creating any difficulty in withdrawing blood samples of 2 ml. each.

Usually, three samples were collected at fifteen-minute intervals. After the forty-five-minute blood sample was removed, glucagon-free insulin† or Na-tolbutamide‡

* 1-Butyl-3-p-tolylsulfonylurea.

Presented at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco on June 22, 1958.

From the Department of Radiology, The Albert Einstein Medical Center, Northern Division, The Department of Chemistry, Temple University, and The Institute for Cancer Research, Philadelphia, Pennsylvania.

*Lucien Moss Home and Clinic of the Albert Einstein Medical Center, Northern Division.

†Kindly supplied by Eli Lilly and Company, Indianapolis, Indiana.

‡Kindly supplied by Dr. C. J. O'Donovan of The Upjohn Company, Kalamazoo, Michigan.

was rapidly injected intravenously through the same indwelling needle. Details of the insulin and tolbutamide dosages are given in the legends of the individual figures. In some of the experiments the insulin was injected subcutaneously into an arm. Blood samples were then removed rapidly (usually at five-minute intervals) for twenty minutes and then every ten minutes for one-and-a-half hours. In the diabetics, urine sugars and acetone were determined at the end of the experiments.* Usually twelve to fourteen blood samples were collected through the same indwelling needle.

RESULTS

Data on the patients used in the six experiments to be reported are given in table 1.

To illustrate the procedure used and to provide a background for the type of information obtained from these experiments, a study of glucose turnover in a nondiabetic individual is described in detail. The patient, an elderly female (F.C.), suffering from posterior lateral sclerosis, was given an intravenous injection of 18 mg. (101 μC.) of glucose, with a specific activity of 12.5×10^6 c.p.m., and blood samples were withdrawn at regular intervals during the ensuing 130 minutes. Results are given in figure 1. Throughout the period the blood sugar remained essentially constant, at approximately 100 mg./100 ml., while the specific activity fell from an extrapolated initial value of 12,000 to 4,800 c.p.m. From the initial dilution of the glucose specific activity, the glucose pool size was estimated to be $12.5 \times 10^6 \times 18/12{,}000 = 18{,}600$ mg. From the average glucose concentration of 100 mg. per 100 ml., we can calculate that the volume occupied by the 18.6 gm. glucose pool was 18.6 liters. This corresponds to $18.6 \times 100/71 = 25$ per cent of the body weight.

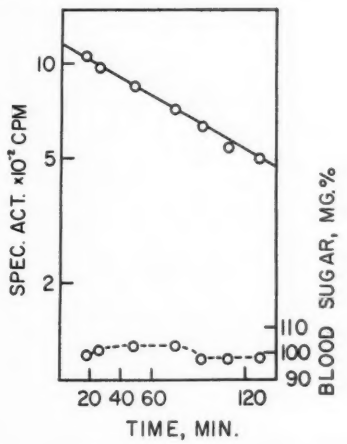

FIG. 1. Blood glucose turnover in a nondiabetic individual (F.C.). Blood sugar level, broken line, right ordinate; specific activity, solid line, left ordinate plotted on a logarithmic scale. Radioactive glucose was administered at time zero.

In figure 1 the logarithm of the specific activity is plotted against time. The linear fall in activity is indicative of a constant rate of replacement of glucose which follows the kinetics of a first order reaction and allows

*At no time was the loss of glucose from the body pool into the urine significant, and ketonuria was never observed.

TABLE 1

Data on patients used in experiments described in figures 1 to 6

Patient	Diagnosis	Sex	Age	Weight	Fasting Blood Sugar	Pool	Glucose Space	Turnover rate
			Years	Kg.	Mg./100 ml.	Gm.	Liters	Mg./100 ml./min.
F.C.	Posterior lateral sclerosis, minimal	F	61	71	100	19	19	0.7
Y.N.	Multiple sclerosis, minimal	F	60	55	100	12	12	1.2
M.R.	Parkinsonism, moderately advanced	M	57	50	90	12	13	0.8
S.I.	ASHD, class III Prostatectomy and orchiectomy for non-metastatic carcinoma of prostate	M	85	66	110	20	16	1.0
A.L.	Diabetes mellitus	M	70	60	250	42	17	1.9
M.W.	Diabetes mellitus	F	68	53	170	19	11	1.6

the calculation of a replacement rate of 0.71 mg. per 100 ml. per minute.[5]

The values of glucose pool size, volume of the pool, and replacement rate thus calculated for all of the experiments here described are listed in table 1. These agree well with those reported by us and others for various species,[6–11] and are typical of values obtained in forty experiments with thirty patients.

EFFECT OF INSULIN ON NONDIABETIC AND DIABETIC HUMANS

Figure 2 shows the results of an experiment in which insulin was administered intravenously to a nondiabetic patient (Y.N.). Again the postabsorptive state was characterized by a logarithmic fall of blood glucose specific activity while the blood sugar level remained constant at 102 mg. per 100 ml. At forty-six minutes, 20 units of glucagon-free insulin was rapidly injected intravenously. The blood sugar began to fall five minutes after injection, and in fifteen minutes it reached the low point of 40 mg. per 100 ml. It rose slowly thereafter, but seventy minutes later it was still depressed. Coinciding with the drop in blood sugar of 62 mg. per 100 ml., there was a clear "plateau" in the specific activity, signifying that glucose was not entering the blood. Coincident with the "leveling off" of the blood sugar concentration, the specific activity resumed its downward trend, and while the blood sugar gradually rose, its specific activity continued to drop at a gradually reducing rate.

Since glucose was not entering the blood during the initial fifteen-minute period of hypoglycemia, we can calculate that the glucose removal rate was (104—42)/15 or 4.1 mg. per 100 ml. per minute. On the basis of a glucose space of 21 per cent in this patient, this equals 8.6 mg. per kilo per minute, which is more than three times the original turnover rate before injection of insulin. The resumption of a downward trend in the specific activity of the blood glucose indicates that after the initial phase of hypoglycemia, glucose again enters the blood, and this is reflected in the return of the blood sugar toward normal. During the period following the plateau, in which the blood sugar is slowly rising and the specific activity is falling, the turnover was calculated by means of Equations Two and Three as described previously.[5] The rates thus calculated are presented graphically in the inset of figure 2. Though the values thus obtained are regarded as only rough approximations, they provide a clear insight into the immediate effects of insulin. One effect is the rise in removal of glucose to three times the original rate. The other immediate response was the complete inhibition of glucose entry. During the recovery period the entry rate was slightly higher than the removal rate; this small difference accounts for the gradual rise toward the normal glucose level. In all aspects these results are virtually identical with those reported previously with normal dogs.[5]

Figure 3 shows essentially the same picture in a diabetic patient (A.L.) given insulin intravenously. The

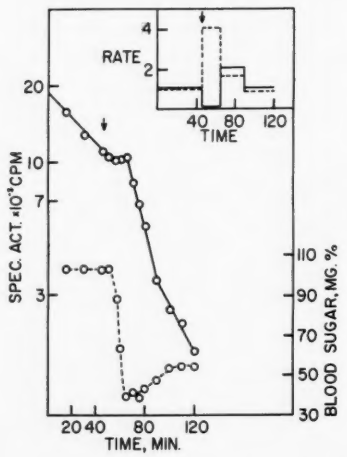

FIG. 2. Time course of blood glucose concentration and specific activity plotted as in figure 1. Arrow indicates time of intravenous administration of 20 units of insulin to a nondiabetic patient (Y.N.) weighing 55 kilos. Inset: Time course of blood glucose entry and removal rates as affected by insulin administration. Entry rate, solid line; removal rate, broken line. The values are given in mg. per 100 ml. per minute.

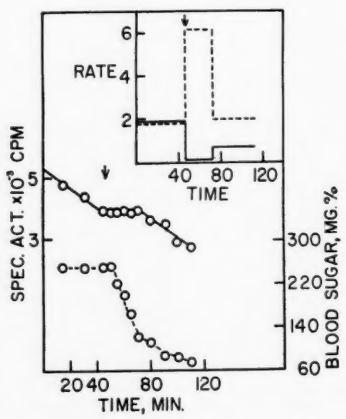

FIG. 3. Same experiment as figure 2, with a diabetic patient (A.L.), weighing 60 kilos and given 6 units of insulin at the arrow.

blood sugar was at a higher level initially, of course, but remained fairly constant, as did the rate of drop in specific activity prior to insulin injection. It is of interest that the postabsorptive turnover rate in this diabetic patient was over twice that of the normal patient of experiment 1. In general, the turnover rates were at least as high, and often higher in the diabetic than in the nondiabetic patients. Five minutes after insulin injection, the blood sugar dropped rapidly, and was still dropping at the termination of the experiment one hour later. During the first eighteen minutes, the blood sugar fell 126 mg. per 100 ml., and the specific activity "plateaued" indicating suppression of glucose entry. Hence the removal rate was $126/18 = 7.0$ mg. per 100 ml. per minute, or about three times the pre-insulin rate. Thereafter, the blood sugar continued to drop, falling to 72 mg. per 100 ml. in the next thirty-eight minutes. During this final period, the specific activity resumed its downtrend, indicating a resumption of glucose entry. The calculated approximate rates of entry and removal are shown in the inset.

EFFECTS OF TOLBUTAMIDE

Figure 4 demonstrates the effect of intravenous tolbutamide in a nondiabetic patient (M.R.). Qualitatively, the pattern of response is strikingly similar to that of insulin. After a ten-minute delay, and exactly coinciding with the period of blood sugar fall, there is a "plateauing" of specific activity. Thus, like insulin, tolbutamide suppresses the output of glucose. The rates calculated for this experiment, shown in the inset, however, demonstrate a remarkable difference between the two substances, in that removal of glucose was not enhanced by tolbutamide. Repeatedly, it has been observed in similar experiments that the entry rate drops to zero but the removal rate does not increase significantly on tolbutamide injection.

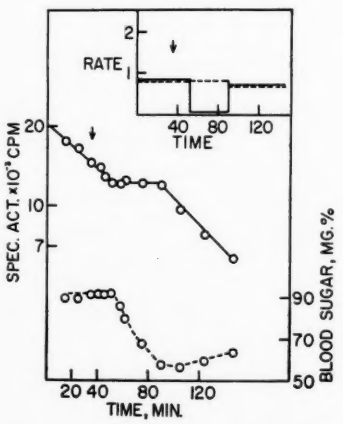

FIG. 4. Blood sugar changes in a nondiabetic patient (M.R.) weighing 50 kilos, given 1.2 gm. Orinase intravenously at the arrow.

Figure 5 demonstrates the effects of intravenous tolbutamide in a diabetic patient (M.W.). Despite the difference in initial glucose level the result is quite similar to the previous experiment. There was initially a logarithmic postabsorptive drop in specific activity, a plateau in specific activity during the hypoglycemic phase following tolbutamide injection, then a resumption of the logarithmic drop in specific activity. Again, the entry was suppressed and the removal rate did not increase.

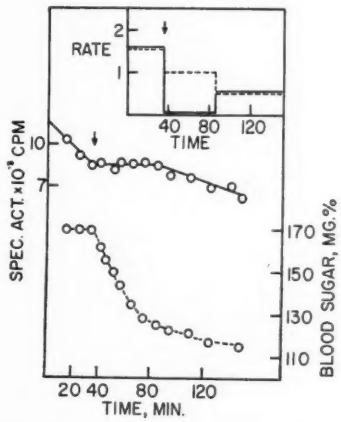

FIG. 5. Same as figure 4, but for a diabetic patient (M.W.) weighing 53 kilos, given 1.0 gm. Orinase intravenously at the arrow.

In every experiment with tolbutamide the same results were obtained. Even when a high dose of 45 mg. per kg. of tolbutamide was rapidly administered intravenously to a normal individual, leading to a mild hypoglycemic reaction, there was no increase in the glucose removal rate.

EFFECT OF SUBCUTANEOUS INSULIN

As discussed previously,[5] one may assume that during fasting essentially all of the glucose which enters the blood comes from the liver, and essentially all which leaves the blood enters peripheral tissues, e.g., brain, muscle, etc. Thus, on this basis, insulin simultaneously and rapidly suppresses hepatic glucose output and stimulates its utilization in peripheral tissues. In contrast tolbutamide, while exerting the former effect, does not appear to influence the latter. At first glance one might assume that this argues against the participation of in-

sulin in the hypoglycemic action of tolbutamide. Indeed, in experiments similar in principle to the present ones, Berson et al.[4] obtained identical results and assumed the Orinase was acting independently of insulin. However, the evidence that tolbutamide acts through insulin, either by eliciting its secretion[12–15] or by preventing its destruction,[16–18] seems hardly disputable. The possibility occurred to us that endogenous insulin, whether elicited physiologically such as during hyperglycemia, or with a drug such as tolbutamide, might exert an hepatic effect but not a peripheral effect.* To test the possibility that a slow, regular introduction of insulin to the bloodstream might give a response different from a rapid intravenous injection, experiments similar to those described above were carried out, but with subcutaneous insulin administration. As shown in figure 6, this hypothesis was clearly confirmed. The results are representative of four such experiments, each on a different individual, and one of whom was a diabetic. Following a normal, fasting blood sugar level and turnover rate, 10 units of insulin was given. Beginning ten minutes later, there was a drop in blood sugar, from the initial level of 105 mg. to a low point of 54 mg. per 100 ml. fifty-two minutes later. During this entire period, there was no decrease in specific activity, indicating a complete suppression of hepatic glucose output. The rate of blood sugar fall thus corresponds to a removal rate of 51 mg. per fifty-two minutes or 1 mg. per minute per 100 ml. This is almost exactly the same as the removal rate prior to insulin injection, and thus clearly demonstrates that insulin, when slowly released to the blood, exerts an hepatic, but not a peripheral action. Another distinguishing feature of subcutaneous insulin administration in these experiments was the unusual lengths of the "plateaus" of specific activity. With intravenous injection of even very large doses, entry rates were not suppressed for longer than twenty minutes, whereas with subcutaneous injection, suppression lasted from forty to sixty minutes.

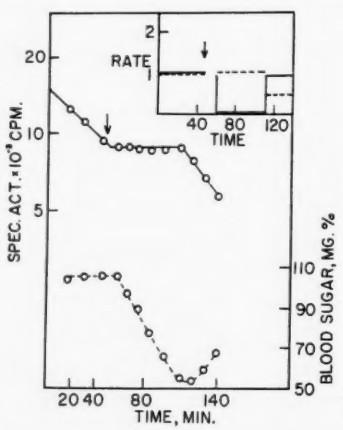

FIG. 6. Effect of 10 units of insulin given subcutaneously to a nondiabetic patient weighing 66 kilos, on the time course of blood glucose concentration and specific activity plotted as in figure 2. Arrow indicates time of insulin administration.

*This possibility has also been considered by Bressler and Engel[19] and has been suggested also by Elrick and Purnell.[20]

DISCUSSION

In agreement with previous work with normal dogs[5] the present study has shown that insulin exerts two immediate effects when given intravenously to diabetic and nondiabetic humans. It completely suppresses the entry of new glucose molecules into the blood and it increases their rate of removal. Since both manifestations have consistently coincided in time with the immediate hypoglycemic action of the hormone, there is little doubt that both are responsible for the hypoglycemia. The significance of these findings to the role of insulin and its mechanism in regulating hepatic glucose output, and to the part this aspect of insulin action may play in clinical diabetes, has been discussed.[5]

In demonstrating that insulin suppresses hepatic glucose output in the human, these results confirm those of Bearn, Billing and Sherlock.[21] By comparing the glucose levels in the blood entering and leaving the liver of normal and diabetic humans, these investigators found an immediate suppression of glucose output following intravenous insulin injection. Recently, Searle et al.[22] also reported, in a preliminary communication, a lowering of hepatic glucose output in the liver of humans immediately following insulin or tolbutamide injection.

On the other hand, Ashmore et al.[23] have recently reported results which are in conflict with the interpretation of the present study. Using an ingenious operative procedure designed to sample hepatic vein blood, these investigators found that tolbutamide diminished hepatic glucose production, but insulin actually increased glucose output. Further support for an increase in hepatic glucose production in response to insulin injection was obtained in C^{14}-glucose turnover studies in rats.

The reason for these discrepancies is not clear; however, in regard to the turnover studies in rats, the suggestion might be offered that, since samples were removed at fifteen-minute intervals, a "plateau" in blood glucose specific activity might have been missed. In some instances we observed a ten-minute "plateau" followed by an accelerated output in our experiments with normal dogs.[5] In a private communication Dr. Ashmore has in-

formed us that they have also observed "plateaus" in blood C^{14}-glucose specific activities of normal dogs following insulin injection.

The hepatic and peripheral effects of tolbutamide have recently been thoroughly reviewed by Stadie.[24] Whenever it has been tested for its action on liver, a suppressive effect on glucose output has been noted.[17,23,26] This action of tolbutamide has been confirmed in the present study, which has also shown that this suppressive effect coincides in time with the hypoglycemic effect and is principally or wholly responsible for the lowering of the blood sugar. The lack of influence of tolbutamide on peripheral glucose utilization noted also by others,[20,28,29] coupled with the virtual certainty that this drug requires insulin for its hypoglycemic action, suggested to us that the physiological action of insulin secreted by the pancreas may also be exerted principally or exclusively on hepatic glucose output. This theory gains plausibility when one considers that the pancreatic secretions drain into the portal vein and must traverse the liver before entering the general circulation. Attempts are being made to test this hypothesis directly by measuring the effect of intraportally administered insulin on the blood glucose turnover of experimental animals.

In the meantime, experiments of the type displayed in figure 6 have clearly shown that the slow release of insulin to the bloodstream following a subcutaneous injection of the hormone has little if any effect on the peripheral utilization of glucose, but causes a sustained suppression of hepatic glucose output.*

For many years the role of the liver in insulin action has been controversial.[cf.32–34] Though recent work from our laboratory has largely discounted a primary role of insulin in liver glycogen storage[25] the present results, coupled with our previous results[8] and those of Bearn et al.[21] suggest that its effect on hepatic glucose output may be more imporant in the physiological regulation of the blood sugar level than its effect on peripheral utilization.

SUMMARY

Diabetic and nondiabetic humans were given a "trace" dose of uniformly C^{14}-labeled glucose and blood samples were removed at frequent and regular intervals before and after administration of insulin or tolbutamide. Before insulin or tolbutamide injection, the logarithmic drop of specific radioactivity coincident with a constant blood glucose concentration indicated a constant rate of replacement of the blood glucose. The "turnover" rates, at 1 to 2 mg. per 100 ml. per min. were at least as high in the diabetic as in the normal subjects. On intravenous insulin injection, there was an immediate, transient suppression of glucose entry and an approximately threefold increased removal rate. With intravenous tolbutamide, glucose entry was suppressed as with insulin, but the removal rate was unaffected. This action of tolbutamide was similar to that of subcutaneously injected insulin, which also caused a suppression of blood glucose entry without affecting its removal. The data are in accord with an action of tolbutamide in stimulating insulin secretion, and are regarded as emphasizing the role of insulin on hepatic glucose output in the physiological action of the hormone.

SUMMARIO IN INTERLINGUA

Action De Insulina E De Tolbutamido Super Le Entrata De Glucosa In Le Sanguine

Humanos diabetic e nondiabetic recipeva doses "traciatori" de glucosa uniformemente marcate con C^{14}, e specimens de sanguine esseva prendite serialmente a intervallos regular, ante e post le administration de insulina o tolbutamido. Ante le injection de insulina o tolbutamido, le descendita logarithmic del radioactivitate specific, in le presentia de constante concentrationes de glucosa sanguinee, indicava un constante intensitate del reimplaciamento de glucosa sanguinee. Le magnitude del reimplaciamento—1 a 2 mg per 100 ml per minuta—esseva al minus tanto alte in diabeticos como in subjectos normal. Post le injection intravenose de insulina, il occurreva immediatemente un suppression transiente del entrata de glucosa insimul con un acceleration approximativemente triple in le processo eliminatori. Post le injection de tolbutamido, le entrata de glucosa esseva supprimite como in le caso del injection de insulina, sed le intensitate del elimination remaneva inalterate. Iste action de tolbutamido esseva simile al action de insulina in administrationes subcutanee, le qual etiam supprime le entrata de glucosa in le sanguine sin afficer le elimination de illo. Le datos se trova de accordo con le conception que tolbutamido stimula le secretion de insulina. Illos es interpretate como apte a relevar le importantia—in le functiones physiologic de insulina—del effecto de iste hormon super le rendimento hepatic de glucosa.

*Miller et al.[30] also noted that the effects of insulin injected subcutaneously on blood pyruvate and lactate levels more nearly resembled the effects of tolbutamide than they resembled those of intravenous insulin. Dulin and Johnston[31] reported that in contrast with rapidly intravenously injected insulin, neither a slow intravenous insulin injection nor tolbutamide increased the muscle glycogen of eviscerated rats. These results also may be explained on the basis of a primarily hepatic action of insulin in low dosage.

ACKNOWLEDGMENT

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Insulin-like Activity of Serum Protein Fractions

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There have been recent studies indicating that specific insulin-like activity is associated with discrete serum protein fractions.[1-3] Insulin-like activity in these studies was determined by a bio-assay technic utilizing blood glucose changes in hypophysectomized-alloxanized mice and rats. Serum protein fractions were obtained by Cohn Methods six, nine, and twelve. The present communication is concerned with further investigations of this subject employing different methods of bio-assay and serum protein fractionation. A new method of assay for insulin-like substances has been devised relating blood glucose decrement of intact 20 to 30 gm. mice to insulin dosage. Serum protein fractions were obtained by preparative electrophoresis performed with a Continuous Flow Electrophoresis Cell.

METHODS

The present method of insulin bio-assay utilized blood glucose decrement of intact 20 to 30 gm. mice as its parameter. Anesthesia was performed by intraperitoneal injection of pentobarbital sodium (Nembutal) in a dosage of 60 to 70 mg. per kg. The initial blood specimen was obtained from the mouse tail and intraperitoneal injection of the test substance was performed simultaneously. The second tail blood specimen was secured one hour later. Blood glucose levels were determined by the Somogyi-Nelson method.[4] Insulin dilutions* were carried out in 5 per cent albumin. Blood glucose values were deleted if the initial blood glucose level was not in the 50 to 150 mg. per cent range.

Serum protein fractionation was performed with a Spinco CP Continuous Flow Electrophoresis Cell. This apparatus operated by permitting a vertical constant regulated flow of buffer and serum downward over a hanging sheet of filter paper which had a horizontal electrical field. The serum fractions, diluted in buffer, flowed from serrated filter paper drip points at the lower border of the hanging filter paper sheet into test tubes. Barbital buffer, *p*H of 8.6 and 0.02 M, was employed. Electrical power of 30 to 60 ma. was supplied by Heathkit, Duostat, or Constat units. The total duration of runs varied from 16 to 36 hr.

Material obtained by this method of preparative electrophoresis was concentrated by dialysis against 20 per cent polyvinylpyrrolidone (PVP) for 2 to 4 hr. One-milliliter volumes of concentrated fractions were assayed in the intact mice. Improved separation of the various serum protein fractions was obtained by previous dialysis of the whole serum against PVP. The greater current of 50 to 60 ma. obtained with the Constat (Spinco) also seemed to favor more satisfactory serum protein electrophoretic separation.

RESULTS

The method of assay employed in this study appears to be sensitive to a minimum of one milli-unit of insulin (figure 1). There is a definite log-dose relationship in

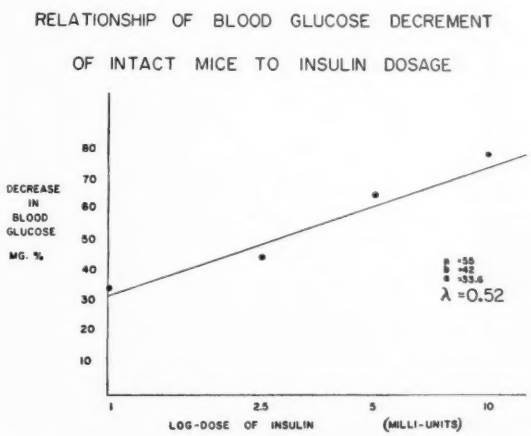

FIG. 1. Relationship between dose of crystalline insulin in 5 per cent albumin and blood glucose decrement (ordinate), calculated one hour following intraperitoneal injection of insulin. Insulin dosage of 1-10 mu. is plotted on log $_{10}$ scale (abscissa). Lambda (index of precision) = s/b, where s is the standard deviation about the regression line and b is the slope of the log-dose line.

*Recrystallized insulin was contributed by the U.S. Pharmacopoeia through the courtesy of Dr. Lloyd Miller.

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From the Department of Medicine, University of Southern California Medical School, and Los Angeles County Hospital, Los Angeles, California.

the one to ten milli-unit range, but, because of the marked standard deviation about the curve, the index of precision, lambda (λ)* is 0.52.

Discrete serum protein fractions were obtainable by this method of preparative electrophoresis (figure 2). The barbiturate buffer employed for the electrophoretic separation was selected as the logical control. Beta globulin possessed predominant insulin-like activity (figure 3, table 1). The mean glucose decrease associated with beta globulin was 32 mg. per cent as compared with 20 mg. per cent for the barbiturate buffer controls, 20 mg. per cent for a component having a mobility intermediate between that of beta and gamma globulins, 19 mg. per cent for alpha globulin, 12 mg. per cent for gamma globulin, and 7 mg. per cent for albumin. The glucose decrement in response to beta globulin was significantly greater than that associated with the barbitu-

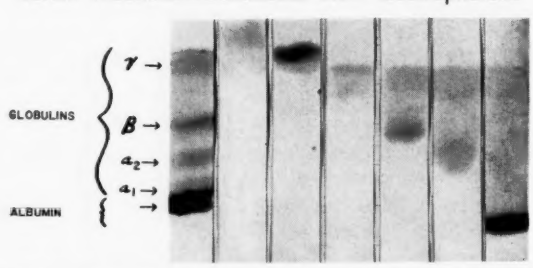

FIG. 2. Delineation by analytic electrophoresis (routine Durrum procedure) of serum protein components of various fractions obtained by preparative electrophoresis (Spinco Continuous Flow Electrophoresis Cell). Strip on left represents analytic electrophoretic pattern of pooled whole serum preceding preparative electrophoresis. Remaining paper strips denote analytic electrophoretic patterns of discrete pools of serum fractions obtained by preparative electrophoresis of the same whole serum. Gamma globulin, a component migrating between gamma and beta globulin, beta globulin, alpha globulin, and albumin each appears to be sole component of successive serum fraction pools (pools composed of contents of one or more of the thirty-two test tubes receiving the fractionated serum).

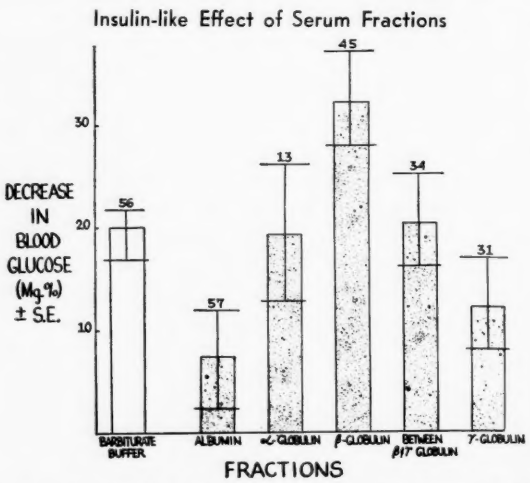

FIG. 3. Effect of discrete serum protein fractions on blood glucose decrement of 20 to 30 gm. intact mice. Mean blood glucose decrement ± standard error of mean (ordinate) calculated as mg. per cent decrease in blood glucose one hour following intraperitoneal injection of 1 ml. of concentrated serum protein fraction or barbiturate control. Numbers above bars represent number of determinations.

* λ = s/b. s is the standard deviation about the regression line; b is the slope of the log-dose line.

TABLE 1

Blood glucose decrement of intact mice in response to serum protein fractions*

Fraction	Number of Determinations	Blood Glucose Decrement (Mg.%) Mean ± S.E.†	Significance of Difference Between Means: P Value Calculated by "T" Test Beta Globulin t	P	Barbiturate Buffer t	P
Beta globulin	45	32 ± 4.6			2.08	>.02,<.05
Alpha globulin	13	19 ± 6.2	1.68	>.05,<.1	0.14	>.8
Gamma globulin	31	12 ± 4.4	3.14	<.01	1.39	>.1,<.2
Between beta and gamma globulin	34	20 ± 4.8	1.81	>.05,<.1	0	>.9
Albumin	57	7 ± 4.4	3.84	<.001	2.20	>.02,<.05
Barbiturate buffer control	56	20 ± 3.7	2.08	>.02,<.05		

*Initial blood glucose level 50 to 150 mg. per cent.
†Standard Error of Mean.

rate buffer control ($P > .02, < .05$), and an even more decisive effect was evident as beta globulin was compared to gamma globulin ($P < .01$) and to albumin ($P < .001$). However, neither alpha globulin nor the component with mobility intermediate between beta and gamma globulins, neither having any appreciable effect on blood glucose decrement as compared with the barbiturate controls, demonstrated statistically significant differences with beta globulin ($P > .05, < 0.1$).

DISCUSSION

The method of insulin assay described above does not possess sufficient sensitivity or reproducibility to constitute an effective insulin assay. However, it is a relatively simple technic and serves as an adequate screening procedure.

Serum protein fractions can be effectively separated in quantity with the Continuous Flow Electrophoresis Cell. Dialysis of the serum against PVP before electrophoresis and employment of a constant, powerful current in the 50 to 60 ma. range appeared to enhance electrophoretic separation.

This study demonstrates that beta globulin has predominant specific insulin-like activity as compared with albumin and gamma globulin. The somewhat increased effect on blood glucose of alpha globulin and of the component migrating between beta and gamma globulins, as compared with gamma globulin and albumin, may be attributable to slight "contamination" by the adjacent beta globulin.

The results of the present investigation support earlier reports that serum fractions containing principally alpha and beta globulins possess insulin-like activity.[1-3] Increased significance may be attached to both these past observations and the present report since quite different methods of serum protein fractionation have been utilized, viz., Cohn Methods six, nine, and twelve in the earlier studies, and electrophoretic separation in the present investigation. Also, the bio-assay procedures differed greatly. The previous technic related diminution of blood glucose increment in hypophysectomized-alloxanized rats and mice, following gavage with dextrin, to insulin dosage. The present method utilized blood glucose decrement of intact 20 to 30 gm. mice as the parameter.

The greater mean glucose decrement associated with the barbiturate buffer controls as compared with albumin and gamma globulin may suggest a nonspecific "insulin-like" effect of the barbiturate. However, the alternative possibility should be considered: that the barbiturate buffer substances may actually constitute the most accurate controls. The diminished insulin-like effect of albumin and gamma globulin as compared with barbiturate buffers may reflect anti-insulin activity of these serum proteins.

It is important to realize that total biological effects are being demonstrated in studies such as these. A number of discrete insulin-like and anti-insulin substances may be involved. Conceivably, some factors totally unrelated to insulin may be operative.

SUMMARY

Serum protein fractions obtained by preparative continuous flow paper electrophoresis were tested by a simplified insulin assay for insulin-like effect utilizing intact mice. The predominant insulin-like effect was associated with beta globulin.

SUMMARIO IN INTERLINGUA

Activitate Insulinoide De Fractiones Proteinic Del Sero

Fractiones de proteina seral, obtenite per electrophorese papiric a fluxo continue esseva testate per medio de un simplificate essayo de insulina pro le presentia de activitate insulinoide in muses intacte. Le predominante effecto insulinoide esseva associate con globulina beta.

ACKNOWLEDGMENT

Portions of this study were aided by Grant No. A-1516, National Institutes of Health, U.S. Public Health Service, and by a grant from the Dazian Foundation.

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Electron Microscopy of Guinea Pig Pancreas

Effect of Cobalt on the Acini and Islets

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The cytomorphologic effects of cobaltous chloride on alpha cells and pancreatic acini of the guinea pig, as revealed by light microscopy, have been described.[1,2,3,4] Distinct vacuolization and degranulation of the alpha cells occurs only after two to three daily injections of cobalt.[3] Van Campenhout[1] reported that partial degranulation and clear spaces near the nuclei occurred in some alpha cells during the first twenty-four to forty-eight hours of treatment but the majority of them appeared normal. Fodden[3] did not find any cytologic changes in the alpha cells during this time.

The purpose of this project was to utilize the higher powers of magnification and resolution obtained by electron microscopy for the detection of possible changes in the ultrastructure of the alpha cells during this initial period. It was hoped that these findings would indicate the origin and mode of formation of the vacuoles as well as provide evidence for either a direct or indirect action of cobalt on the alpha cells. This study was feasible since the electron microscopic differentiation[5] and characterization[6] of normal islet cells of the guinea pig had been accomplished previously.

MATERIALS AND METHODS

Seventeen guinea pigs of both sexes weighing 300-425 gm. were used. Three served as controls. The remainder were injected subcutaneously daily with 25 mg. per kg. of body weight of cobaltous chloride as a 0.5 per cent solution in saline. On each of five consecutive days, two of the animals were killed starting twenty-four hours after the first injection, and at six and thirteen days one each was killed. In two animals, the daily injections were stopped after five days and they were killed eight days after the last injection.

The method of preparing the tissue for electron microscopy was essentially the same as described previously.[5,6] A portion of the tail of the pancreas was removed immediately after the death of the animal and placed in a few drops of 1 per cent osmic acid-dichromate solution. The tissue was cut into small (1 mm.) pieces with a sharp razor blade while immersed in the fixative. They were then transferred to a 1 per cent osmic acid-dichromate solution buffered to a *p*H of 7.6[7] and were fixed for one hour at room temperature. They were dehydrated with a graded series of ethanol solutions and embedded in a mixture of eight parts butyl and one part methyl methacrylate. The methacrylate was polymerized at 60° C. for twenty-four hours and benzoyl peroxide was used as a catalyst. A uniform schedule was used for fixation, dehydration and embedding all of the pancreatic tissue in order to decrease the possibility of artifacts occurring from variations in these procedures. Sections of the pancreas were cut on a Servall microtome using glass knives. The islets were localized within the blocks of pancreas by examining relatively thick sections (2-3 μ) with a phase microscope. Thin sections of the islets and pancreatic acini were examined in an RCA electron microscope (EMU 3B) without removing the plastic. The electron micrographs were taken at original magnification of 2,000 to 6,000 diameters and enlarged photographically.

A portion of pancreas from each animal was also fixed in Bouin's fluid for eighteen to twenty-four hours, dehydrated and embedded in paraffin for light microscopic examination. The paraffin sections were stained with hematoxylin and eosin, chrome alum hematoxylin,[8] aldehyde fuchsin[9] and the periodic acid Schiff reaction for glycogen.[10] A portion of pancreas was also fixed in cobalt-formol and frozen sections of this were stained with Oil Red O[11] and Sudan Black B for lipid.

OBSERVATIONS

Alpha cells. Definite vacuolization was apparent by light (figure 1) and electron microscopy (figure 2) after seventy-two hours of treatment. Prior to this time, neither degranulation nor vacuolization was apparent by electron microscopy. This observation was confirmed by light microscopy with the exception of one animal in which a few vacuolated alpha cells were observed at forty-eight hours.

The large vacuoles were usually limited by a definite membrane (electron micrograph, figure 2) and they contained a small amount of grey, amorphous material. The concentration and density of this material were less than the coagulated proteins observed within the capillaries. It did not react positively to histologic tests for

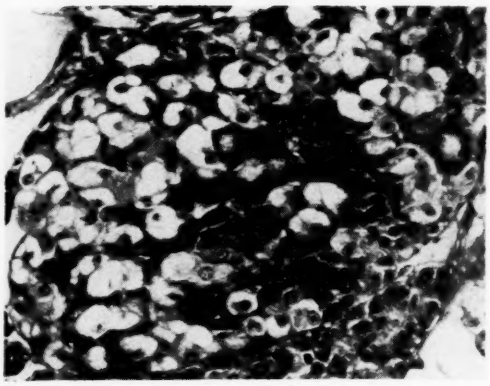

FIG. 1. Photomicrograph of vacuolated alpha cells after three days of treatment with cobalt. Chrome alum hematoxylin and phloxine stain. X 275.

either glycogen or lipids.

The cytoplasm between the vacuoles contained mitochondria, granules, ergastoplasm and portions of the Golgi apparatus (figure 2). In some of the vacuolated alpha cells, the mitochondria were dilated and contained an amorphous, grey material. In others, the mitochondria appeared normal in size and density (figure 3). Granules were present in the intervening cytoplasm but the number per unit of area was less than in the normal alpha cell (figure 3). Complete degranulation of vacuolated alpha cells was not observed even after thirteen days of treatment.

In some cells, the vacuoles were numerous and small, approximately the size of mitochondria (figure 4). The membranes surrounding them had small cytoplasmic granules on their outer surfaces. These vacuoles appeared to originate from the ergastoplasm since it is composed of membranes and associated small granules.[12] Further dilatation and possibly fusion of these ergastoplasmic sacs could have led to the formation of the large vacuoles illustrated in figure 2. Partial degranulation was also apparent in those cells containing small vacuoles. Frequently, normal alpha cells were observed adjacent to vacuolated and partially degranulated cells (figure 3). The nuclei of the vacuolated cells appeared normal and contained nucleoli. The nuclear and plasma membranes were intact. Lipid droplets were frequently observed in both vacuolated and nonvacuolated alpha cells (figure 3). They are rarely found in the normal alpha cell of the guinea pig.

The only alteration in the ultrastructure of the beta and C cells was the presence of occasional lipid droplets in their cytoplasm. The capillary endothelium and their associated basement membranes were normal.

The electron microscopic changes observed in the alpha cells were reversible. Vacuolization, degranulation and lipid droplets had completely disappeared eight days following the cessation of injections with cobalt.

Acinar cells. Three distinct changes were apparent in the acinar cells twenty-four hours after the first injection of cobaltous chloride. The first was partial degranulation and a decrease in the size of the acinar cells, evident by both light and electron microscopy. Figure 5 illustrates a normal acinar cell with large zymogen granules near its apex while a partially degranulated cell is shown in figure 6. The second was the presence of large, vacuolated mitochondria in some of the partially degranulated cells (figure 6). The cristae appeared clumped or as isolated remnants within the grey, amorphous material filling the mitochondria. This degenerative change was not uniform since mitochondria of normal size and structure were observed in the same cells containing the vacuolated forms. The third change was the frequent presence of lipid droplets in the acinar cells (figure 7). Lipid droplets are rarely observed in normal acinar cells of the guinea pig.[13]

Further treatment with cobaltous chloride increased the severity and frequency of the changes observed at twenty-four hours. Nearly all of the large zymogen granules disappeared from the cells after the fourth or fifth day. Degranulation did not become complete since the acinar cells now contained numerous small granules which were approximately the size of beta granules (figure 8). Clusters of two to four of these granules were frequently observed in the same ergastoplasmic sac. Zymogen granules of this size are observed occasionally in the basal portion of acinar cells of the normal guinea pig.[13] Enormous, nonvacuolated mitochondria were present in some of these cells (figure 8). Their diameters were more than twice the average diameter of normal mitochondria. They contained distinct cristae which traversed their entire width in many instances. The number and size of the lipid droplets increased (figures 7 and 9) and they were apparent in frozen sections of the pancreas stained with Sudan Black B (figure 10).

Focal areas of degeneration occurred in the cytoplasm after five to six days of treatment. They appeared as either foci of dense, granular material or as whorled, laminated structures (figure 9). A distinct membrane surrounded some of the areas of granular debris. These cells also contained large lipid droplets, giant mitochondria and numerous ergastoplasmic sacs containing small zymogen granules (figure 9).

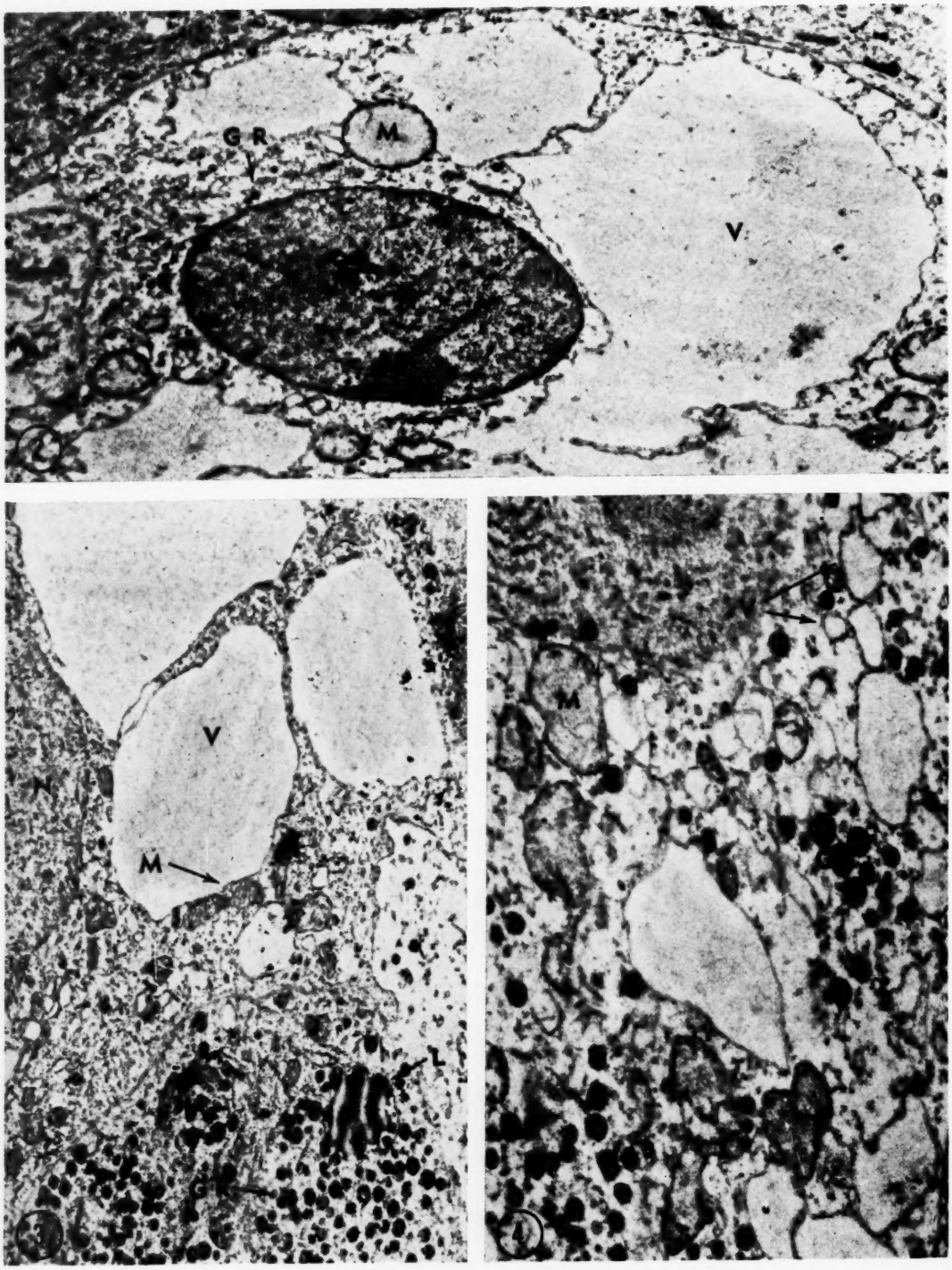
GR
M
V
V
M
M
V
L

FIG. 2. Large vacuoles (V) containing a small amount of grey, amorphous material are present in this alpha cell after five days of cobalt treatment. A distinct membrane surrounds each of the vacuoles. The mitochondria (M) are dilated. A few granules (GR) are present in the cytoplasm between the vacuoles. The nucleus (N) and its nuclear membrane appear normal. X 9,000.

FIG. 3. The portion of the alpha cell in the upper part of the electron micrograph contains large vacuoles (V) but its mitochondria (M) are normal in size and structure. The nucleus (N) is normal. The adjacent alpha cell appears normal except for a lipid droplet (L) in its cytoplasm. The lipid has an irregular contour and it is not limited by any definite membrane. Numerous granules (GR) of normal size and density are present. Four daily injections of cobalt had been given. X 13,200.

FIG. 4. A portion of an alpha cell containing small vacuoles (V) after four days of cobalt treatment is illustrated. The membranes surrounding these small vacuoles have cytoplasmic granules on their outer surface similar to the normal ergastoplasm. The vacuoles contain a grey, amorphous substance. Granules (GR) are present in the intervening cytoplasm. The mitochondria are dilated. Portions of the cristae are evident near the outer, smooth limiting membranes of the mitochondria. X 18,000.

The electron microscopic appearance of the acinar cells was almost normal eight days after the cobalt injections were stopped. The mitochondria were normal in size and structure. Numerous small granules were present in the base of the acinar cells and large zymogen granules were found at their apex. Lipid droplets and debris were observed in numerous macrophages present in the interstitial spaces. An occasional acinar cell contained lipid droplets and small foci of degeneration.

DISCUSSION

The earliest electron microscopic change was found in the pancreatic acini instead of the alpha cells. Partial degranulation, lipid droplets and degeneration of the mitochondria were present at twenty-four hours. Lison and Valeri[14] found a partial or total degranulation of acinar cells as early as ten to twelve minutes after an intravenous or intraperitoneal injection of cobalt. The small granules which persisted in the acini (figure 8) represent either a stage in the formation of zymogen granules or a type different from the large zymogen granules.[13] They are not beta granules since their electron microscopic structure was different from the normal, irregularly shaped ones of the beta cell and they did not stain with aldehyde fuchsin. After four to five days of treatment, they were the only type of granules observed in nearly all of the acinar cells. We did not observe any evidence of new islets forming from acinar cells as described by Van Campenhout.[15]

The mitochondria of the acinar cells were the organelles most severely affected by cobalt. Vacuolization and degeneration occurred in only a portion of their mitochondria. This may explain the lack of acute necrosis of these cells. The enormous mitochondria which formed may be hypertrophied normal ones but their exact origin and significance can not be determined until further knowledge of the origin, development and fate of normal mitochondria is obtained. It was not possible to determine whether cobalt was selectively concentrated by the mitochondria. Dense granules were observed in the degenerating and enlarged ones but similar particles were observed in normal mitochondia of controls.

The lipid droplets which have been described previously following treatment with cobalt[16,2] did not appear to form from the degenerating mitochondria. Initially, they were observed in the basal portion of the acinar cells where they are found occasionally in the normal guinea pig.[13] They have also been observed with electron microscopy in the pancreatic acini of rabbits treated with alloxan.[17] Further studies are being made on the occurrence of lipid in acinar cells after treatment with Synthalin and glucagon in an attempt to elucidate further their mode of formation and significance.

The sequence of events leading to vacuolization of the alpha cells was difficult to determine because of the delay in the onset of vacuolization and the absence of electron microscopic changes during this time. The vacuoles apparently originated from dilated ergastoplasmic sacs since the membranes surrounding the small vacuoles had cytoplasmic granules on their outer surfaces similar to normal ergastoplasm. The small amount of electron dense material present in the fluid of the vacuoles was probably a protein since histochemical stains for lipid and glycogen were negative both in this study and in others.[2,18] Changes in the mitochondria of the alpha cells were not as distinct as in the acinar cells. Dilated mitochondria were observed in some of the vacuolated cells while others contained normal ones. Scattered alpha cell granules were still present even in the markedly vacuolated forms.

Fodden[3] reported a similar delay of forty-eight to seventy-two hours before vacuolization and degranulation occurred in the alpha cells of guinea pigs, even though the daily doses of cobaltous chloride were greater than the amounts used in this study. Creutzfeldt[19] has suggested that this delay may indicate a secondary degeneration in the alpha cells. Another possibility is that alpha cells have less affinity for cobalt than acinar cells and a longer period of treatment is needed to produce changes in the alpha cells. The early appearance of elec-

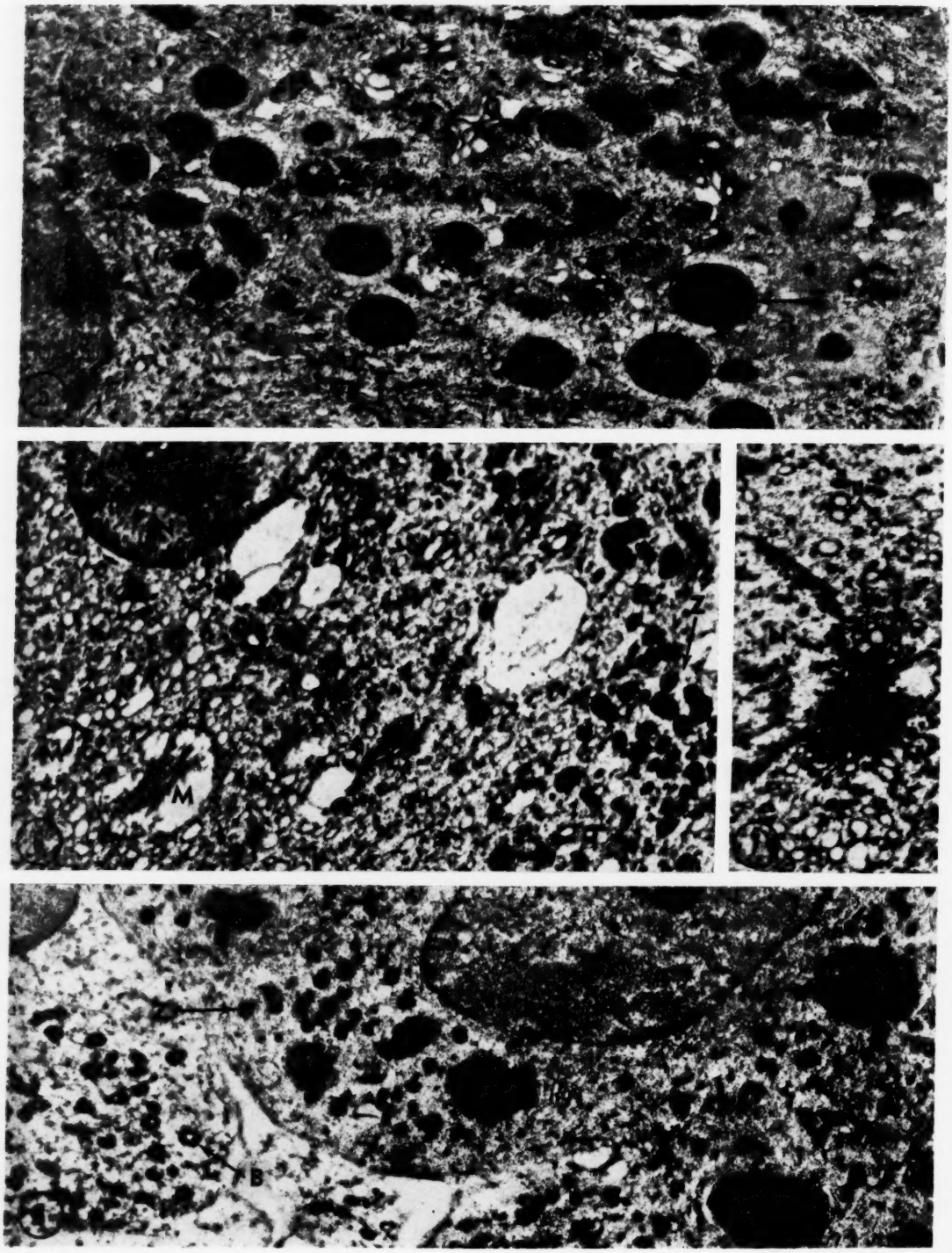
M
M
B

FIG. 5. Electron micrograph of a portion of a normal acinar cell. Large zymogen granules (Z) are present near the apex of the cell. Mitochondria (M) are scattered throughout its cytoplasm. The ergastoplasm (ER) is abundant. G = Golgi apparatus. N = nucleus. X 12,000.

FIG. 6. Partial degranulation of this acinar cell is apparent twenty-four hours after one injection of cobalt. The zymogen granules (Z) are smaller than normal and are clustered around the lumen of the acinus. The mitochondria (M) are dilated and their cristae are clumped. The nucleus (N) appears normal. X 13,000.

FIG. 7. A lipid droplet (L) is shown in the cytoplasm of an acinar cell twenty-four hours after the first injection of cobalt. N = nucleus. X 12,000.

FIG. 8. Mitochondria (M) with diameters much greater than the normal (figure 5) are present in these acinar cells after four days of cobalt treatment. These enormous mitochondria are not vacuolated and the cristae traverse their entire width in some instances. Only small zymogen granules (Z) are present in these cells. A portion of a beta cell is shown in the lower left hand corner. The beta granules (B) are irregularly shaped and clear areas are present in the center of some. The small zymogen granules (Z) have a uniform density with smooth, round contours. N = nucleus. X 11,600.

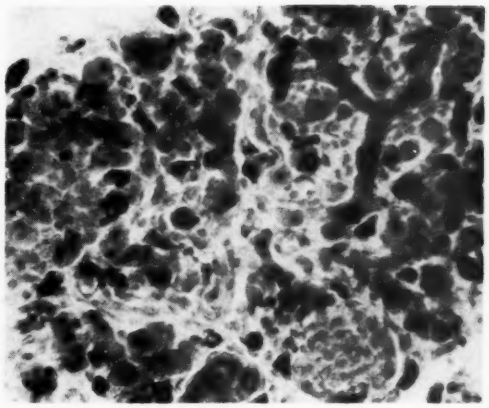

FIG. 10. Lipid droplets are shown in the acinar cells and macrophages after thirteen days of treatment with cobalt. Sudan Black B with Feulgen stain. X 275.

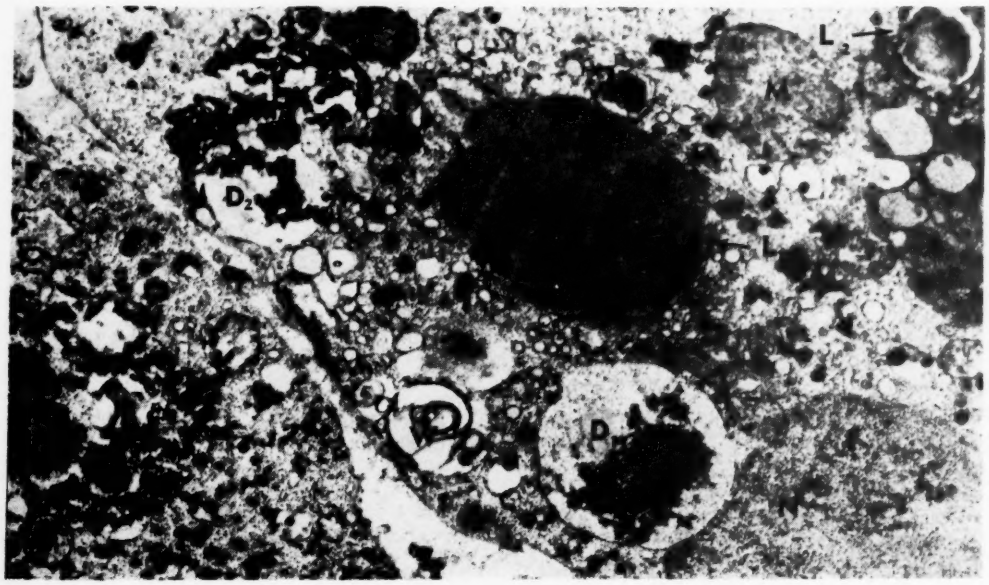

FIG. 9. Focal areas of degeneration of the cytoplasm are shown in the acinar cell after six days of cobalt treatment. These foci appear as either granular debris (D_1) or whorled, laminated structures (W). A membrane surrounds one focus of granular debris (D_1) and is absent in another (D_2). Enormous mitochondria (M) are present. The cell contains a large lipid droplet (L_1) and a smaller one (L_2). The nucleus (N) appears normal. X 13,000.

tron microscopic changes in acinar cells and the later occurrence of changes in alpha cells is consistent with either hypothesis. A comparison of the electron microscopic appearance of vacuolated alpha cells produced by cobalt and hydropic or glycogen-infiltrated beta cells produced by cortisone may help to determine whether the change in the alpha cells is primary or secondary.

SUMMARY

The effects of cobaltous chloride on the ultrastructure of the acini and islet cells of the guinea pig pancreas were studied. Partial degranulation, lipid droplets and degenerating mitochondria were observed in the acinar cells before any alteration of the ultrastructure of the alpha cells occurred. The large zymogen granules progressively disappeared from the acinar cells and only the small type of zymogen granule persisted. The initial change in some of their mitochondria was vacuolization and clumping of the matrix. Later, enormous mitochondria were present as well as normal and degenerating forms in some of the acinar cells. The lipid droplets did not appear to originate from the degenerating mitochondria.

Electron microscopic evidence of vacuolization and degranulation was not apparent in the alpha cells until after three daily injections of cobaltous chloride, 25 mg. per kg. of body weight daily. The vacuoles apparently originated from dilated ergastoplasmic sacs. The cytoplasm between the vacuoles contained normal or dilated mitochondria, scattered alpha cell granules, ergastoplasm, lipid droplets and portions of the Golgi apparatus. Complete degranulation of alpha cells was not observed even in the most vacuolated forms. These electron microscopic changes reverted to normal following cessation of treatment with cobaltous chloride.

SUMMARIO IN INTERLINGUA

Microscopia Electronic Del Effecto De Cobalt Super Le Acinos E Le Insulas Del Pancreas De Porcos De India

Esseva studiate le effectos de chloruro cobaltose super le ultrastructura del acinos e del cellulas del insulas in le pancreas de porcos de India. Disgranulation partial, guttettas lipidic, e mitochondrios degenerante esseva observate in le cellulas acinar ante le occurrentia de ulle alteration del ultrastructura in le cellulas alpha. Le grande typo de granulos zymogenic dispareva progressivemente ab le cellulas acinar e solmente le micre typo persisteva. Le alteration initial in certes del mitochondrios esseva vacuolisation e glutination del matrice. Plus tarde, mitochondrios de dimensiones enorme e etiam de forma normal e de forma degenerante esseva presente in certes del cellulas acinar. Il pareva que le guttettas lipidic non habeva lor origine in le degeneration del mitochondrios.

Le microscopia electronic revelava nulle signos de vacuolisation e de disgranulation in le cellulas alpha usque post tres injectiones per die de chloruro cobaltose in doses de 25 mg per kg de peso corporee. Le vacuolos prendeva lor origine apparentemente in dilatate saccos ergastoplasmic. Le cytoplasma inter le vacuolos contineva mitochondrios normal o dilatate, disperse granulos de cellula alpha, ergastoplasma, guttettas lipidic, e portiones del apparato de Golgi. Disgranulation complete de cellulas alpha non esseva observate mesmo in le formas le plus vacuolate. Iste alterationes in le aspecto de microscopia electronic se reverteva al stato normal post cessation del tractamento con chloruro cobaltose.

ACKNOWLEDGMENT

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Mechanism of Impaired Glucose Tolerance in Uremia and Experimental Hyperazotemia

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The influence of intercapillary glomerulosclerosis with azotemia on the clinical course of diabetes mellitus is uncertain. Zubrod et al.[1] reported that patients who developed this complication of diabetes mellitus required less insulin for adequate management after the onset of intercapillary glomerulosclerosis than they did early in the course of their disease. On the other hand, Runyan et al.[2] and Epstein and Zupa[3] found that the pathologic lesion observed at autopsy in the kidneys of diabetic patients bore little relationship to the clinical course of the disease and that the insulin requirement during azotemia was increased as often as it was decreased. The latter authors felt that nonspecific factors related to dietary intake, weight loss, and nausea and vomiting in these severely ill patients were responsible for the changes observed.

Quantitative studies of carbohydrate metabolism in azotemia are few in number and such information as is available has not been considered in relation to the effect of renal functional impairment in diabetes. Hamman and Hirschman, in 1917,[4] performed oral glucose tolerance tests on six patients with renal disease, azotemia and decreased excretion of phenolsulfonphthalein. They found slight elevation of the fasting blood sugar and a diabetic-type glucose tolerance curve in each instance. Linder et al.[5] reported the results of fifteen glucose tolerance tests in thirteen patients with renal disease. Diabetes-like glucose tolerance tests were observed in those patients with advanced disease and azotemia, although no correlation with the level of the blood urea was apparent. Myers and Bailey[6] also observed elevated fasting blood sugars in some of their patients with uremia.

The purpose of the present study was twofold: first, to gain information that might clarify the clinical controversy over the course of diabetes mellitus in azotemic patients; and second, to confirm and extend the earlier observations on glucose tolerance in azotemia. Studies have been made of the mechanisms responsible for the observed changes.

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MATERIALS AND METHODS

Twenty-three patients (four females, nineteen males) with uremia due to various causes have been studied. These were unselected, serial admissions to the University of Utah teaching hospitals except that patients with previous histories of findings of diabetes mellitus were excluded. The pertinent clinical data are summarized in table 1. For the purposes of this study, a blood urea nitrogen level of 30 mg. per 100 ml. or higher was considered to represent azotemia. Each patient received a minimum of 200 gm. carbohydrate orally or parenterally for at least forty-eight hours prior to testing in order to minimize the influence of starvation upon glucose tolerance. Patients with central nervous system disease were excluded because of the well-known effects of brain damage on carbohydrate tolerance; preterminal patients were likewise excluded from these studies. None of the subjects had glycosuria or ketonuria. Six had been observed previously for periods of from one to seven years and no abnormality of carbohydrate metabolism had been noted. Diabetes mellitus was excluded clinically in the other seventeen patients on the basis of the nonprogressive nature of the abnormality in carbohydrate metabolism and the remainder of their clinical course.

Twenty-eight control subjects have been studied. These have included medical students, house officers, nonprofessional hospital employees and patients mildly ill with a variety of chronic illnesses but having normal blood urea nitrogen levels. Of this group, nine were females, ages twenty to fifty-two, and nineteen were males, ages fifteen to eighty-two.

Standard 100 gm. oral glucose tolerance tests were performed on seven control subjects and ten azotemic patients. Intravenous glucose tolerance tests, 0.5 gm. glucose per kg. body weight administered as a 20 per cent solution by constant intravenous drip over a thirty-minute period, were performed on eleven controls and on seven azotemic patients. Glucose-insulin tolerance tests were carried out on nine controls and fifteen azotemic

TABLE 1

Uremic patients, clinical and accessory data

Name	Age	Sex	Blood Urea Nitrogen mg./100 ml.	Creatinine mg./100 ml.	Plasma 17-Hydroxycorticosteroids γ/100 ml.	Serum Sodium mEq./L.	Serum Potassium mEq./L.	Diagnosis
FSC	93	M	41.5	2.8				Benign prostatic hypertrophy
RF	37	M	90	15.6		128	4.5	Chronic glomerulonephritis
BG	80	M	89	8.7				Carcinoma of prostate with metastases to the bladder
F	83	M	29	2.3				Benign prostatic hypertrophy
WO'B	45	M	74	3.1				Chronic glomerulonephritis Subacute bacterial endocarditis
CM	52	M	45	2.8		139	5.4	?
JG	84	M	61	4.4				Benign prostatic hypertrophy
PG	68	M	125	15.1		140	3.7	Multiple myeloma
CS	43	F	88			127	5.2	?
AG	41	M	77	8				Chronic pyelonephritis
TMcL	70	M	90	8.1	24			Carcinoma of the bladder
VS	28	F	132	14.9	12	152	5.5	Chronic pyelonephritis
RC	51	M	132	7.6		134	4.8	?
P	22	M	80		24	137	4.2	Chronic glomerulonephritis
C	64	M	40		24	116	4.2	Chronic pyelonephritis
LS	74	F	56		27			Chronic pyelonephritis
PM	63	M	45		30			Nephrosclerosis
TC	48	M	95	6.6				Chronic pyelonephritis
PM	65	M	39	3.0	13			Subacute bacterial endocarditis
EB	63	M	117	6.3	27.2	145	3.8	Vascular nephritis
MT	76	M	111	3.0	14.2			Urine ingestion Senile psychosis
JD	50	M	111	16.5		135	5.9	Nephrosclerosis
IH	62	F	47					Chronic pyelonephritis

patients according to the technic of Volk et al.[7,8] In this test, 25 gm. of glucose are given intravenously as a single injection. After thirty minutes, a blood sample is drawn and 0.1 unit regular insulin per kilogram body weight is given intravenously. The blood sugar is measured at ten-minute intervals thereafter for thirty minutes, with a final sample one hour post-insulin.

In addition, standard intravenous glucose tolerance tests were performed, as described, on three normal male medical students before and after the ingestion of 60 gm. of urea per day by mouth in four divided doses for three and one-fourth days. The subjects' food intake remained good during the period of urea ingestion and included more than 250 gm. carbohydrate. Multiple sampling of venous blood was accomplished by the use of an indwelling needle kept patent with a heparinized stylet.

Studies of the influence of urea on the glucose tolerance of dogs have also been made. Urea, 2 gm. per kilogram body weight, was administered intravenously to mongrel dogs. This dose, which was adequate to raise the blood urea nitrogen levels to 100 mg. per 100 ml., was followed immediately by a single injection of glucose (1 gm. per kilogram body weight). Samples of blood were drawn at five- to ten-minute intervals for sixty minutes for glucose analysis. The blood level of urea did not decrease appreciably during the course of the test, but was always back to control levels within twenty-four hours. Glucose tolerance tests were also performed in dogs after intravenous injection of sodium chloride, 1 gm. per kilogram, a dose osmotically equivalent to the urea loads, and after injections of creatinine doses adequate to raise serum creatinine levels to 10 mg. per 100 ml. For studies with sodium chloride and creatinine, glucose dosage was 0.5 gm. per kg. body weight.

In addition, the effect of urea and creatinine on the uptake of glucose by rat diaphragm was measured in vitro. Male Sprague-Dawley rats (100 to 150 gm.) were killed by decapitation and the diaphragms were removed, trimmed and chilled. Weighed portions of diaphragms from four rats were placed in Warburg flasks containing 2 ml. Krebs-Ringer bicarbonate buffer with a glucose concentration of 200 mg. per 100 ml. The gas phase was oxygen, 90 per cent, carbon dioxide, 10 per cent. Insulin, 0.2 units per ml., was used in all insulin flasks, and the urea and creatinine concentra-

tions used were 100 mg. per 100 ml. and 10 mg. per 100 ml., respectively. The incubation time was ninety minutes and glucose uptake was determined by the difference in glucose concentration in the buffer before and after incubation.

The effect of these concentrations of urea and creatinine on insulinase activity in rat liver extracts was also studied. Crystalline insulin* was iodinated with I^{131} according to the technic of Ferrebee et al.[9] and incubated with rat liver extract as described by Mirsky, et al.[10] The radioactivity released from the insulin was measured in a well-type scintillation counter.

Glucose was determined by the Nelson-Somogyi method.[11] Sodium and potassium were measured with a Beckman flame photometer. Blood urea nitrogen was measured by the technic of Ormsby.[12] Creatinine was determined by a modification of the method of Phillips.[13] Seventeen-hydroxycorticosteroids were measured by the Nelson-Samuels method.[14]

METHODS OF ANALYSIS OF GLUCOSE TOLERANCE DATA

Values for blood glucose obtained in the oral glucose tolerance tests were plotted on ordinary graph paper as the mean change from fasting level ± one standard deviation of the mean† (figure 1). The intravenous glucose and glucose-insulin tolerance tests have been evaluated in a different manner. Most authors[7,8] have used the average decrease in blood glucose (mg. per 100 ml. per minute) as a measure of glucose disappearance. However, our graphs of the blood glucose concentrations in these tests yielded parabolic curves. When the same data were plotted as the logarithm of the blood glucose value against time, a straight line was obtained, demonstrating that the glucose disappeared from the blood stream in an exponential fashion. Silverstone et al.,[15] Amatuzio et al.,[16] and Marks and Bishop[17] have reported the same observation. Analysis of the graphs of intravenous glucose tolerance plotted logarithmically readily yields a constant (−k, per cent fall in blood glucose per minute) which is a reproducible expression of the disappearance of glucose from the blood. The fit of the curves is essentially the same whether absolute blood glucose values or the increment over the fasting levels is used.[17] Accordingly, for the graphs depicting glucose disappearance in human subjects and for the data obtained in the dog glucose tolerance tests without pretreatment and after pretreatment with urea or sodium chloride, the logarithms of the observed blood glucose values in each subject were plotted around the line of best fit obtained by the method of least squares[18] and the K value was calculated by conversion of the blood glucose data to the logarithmic form using the equation $K = 2.3 \times \log_{10} \frac{A_i}{A_f} \Big/ T^1$, where A_i equals the initial blood glucose concentration in mg. per 100 ml., A_f equals final blood glucose concentration in mg. per 100 ml. and T^1 is the time in minutes between A_i and A_f. A_i and A_f were determined graphically from the lines of best fit. Similar methods were used for the data obtained in the three normal subjects given urea orally. Standard statistical methods for evaluation of small samples were applied to the data.[18]

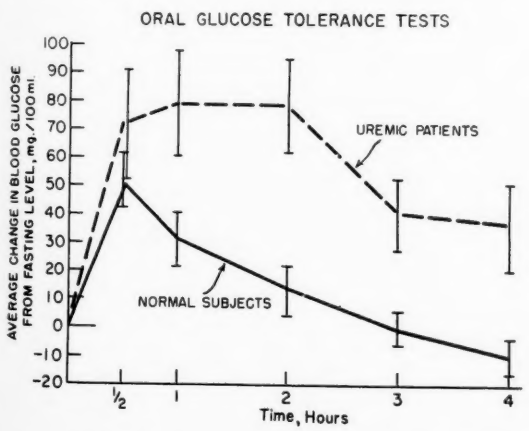

FIG. 1. This graph demonstrates the mean values in the oral glucose tolerance tests in control and uremic patients. Values on the abscissa indicate mg. per 100 ml. change in blood glucose levels as compared with those found while fasting. The bars about each point depict ± one standard error of the mean for each set of values.

As the work described in this paper was in progress, observations were published[15] demonstrating that the rate of glucose disappearance from the blood stream decreases with increasing age. Analysis of our control series showed that most of our subjects were under sixty years of age. Therefore, in addition to using our own control data where it was applicable, we have depicted the results of the intravenous glucose and glucose-insulin tolerance tests according to age together with the comparable age-selected controls published by Silverstone et al.[15] Although certain differences exist between the methods used for the glucose and glucose-insulin tolerance tests in the present study and that of Silverstone et al., the use of their controls is considered justified for three rea-

*Kindly supplied by W. R. Kirtley, M.D., Eli Lilly and Company, Indianapolis, Indiana.

† Standard deviation of the mean: $\sqrt{\frac{\Sigma(x-\bar{x})^2}{N}}$

sons. First, it can be assumed that mixing of the administered glucose with the blood is adequate after a thirty-minute constant infusion. Second, the dose of glucose used is similar in the two studies. Third, the control values obtained in age-comparable subjects are the same by both methods.

RESULTS

1. *Fasting Blood Glucose.* The fasting blood glucose level in the control subjects was 78 ± 13 mg. per 100 ml. and in the azotemic patients 92 ± 19.1 mg. per 100 ml. ($t = 2.12$). The difference is significant at the 5 per cent level but not at the 1 per cent level of confidence.

2. *Oral Glucose Tolerance Tests.* The results of the oral glucose tolerance tests are shown in figure 1. The abnormal character of the curve for the uremic patients is evident. The peak values obtained after glucose administration were higher in these patients and the blood sugars were still elevated after four hours. The differences are statistically significant at the 5 per cent level of confidence at two, three and four hours in this small series.

3. *Intravenous Glucose Tolerance Tests.* Figure 2 depicts the results of the intravenous glucose tolerance tests.

The log of the glucose concentrations is plotted against time for the uremic patients and against the comparable age-selected control subjects of Silverstone et al.[15] Here the differences between the two groups are more apparent. The K values for the calculated lines of best fit are: uremics over sixty, 0.77 per cent per minute; controls over sixty, 0.98 per cent per minute;[15] uremics under sixty, 0.83 per cent per minute; controls under sixty, 1.44 per cent per minute.[15] These data are shown in table 2 together with our controls for two groups of patients under sixty years of age. These values are all significant at less than 1 per cent level of confidence, except for the uremics over sixty. In this small number of cases, the results are not statistically significant.

4. *Glucose-Insulin Tolerance Tests.* After the intravenous administration of a single injection of glucose, the blood sugar levels showed a greater rise in all azotemic subjects than in the controls. After the insulin was given, the blood sugar levels in the control group fell rapidly to reach a nadir in thirty minutes and then rose. In the uremic patients, the rate of fall was considerably slower, and the blood sugar was still decreasing one hour after insulin administration. These data, which indicate decreased action of insulin in the uremic patients, are shown in figure 3, together with those obtained in age-comparable subjects.[15] The K values (table 2) are: uremics over sixty, 1.39 per cent per minute;

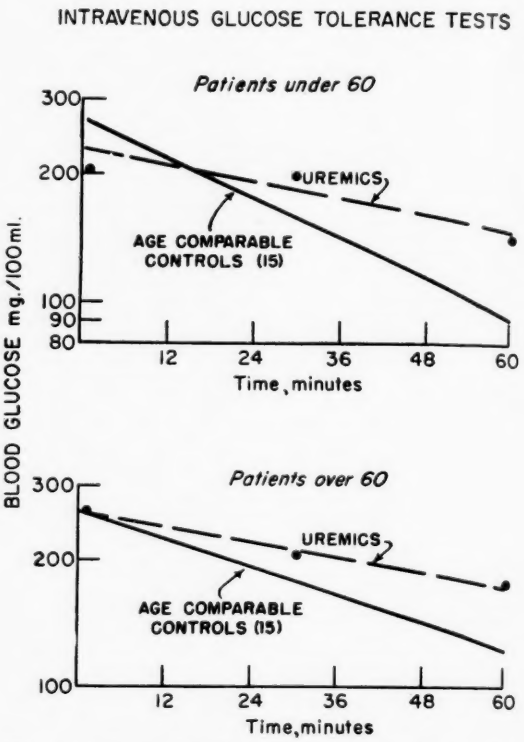

FIG. 2. Intravenous glucose tolerance tests in uremic patients and age comparable controls.[15] The upper portion of this and subsequent figures depicts the results in the age group under sixty; the lower portion depicts the results in the age group over sixty.

controls over sixty,[15] 2.49 per cent per minute; uremics under sixty, 1.47 per cent per minute; controls under sixty,[15] 3.61 per cent per minute. These values are significantly different at less than the 1 per cent level of confidence.

Statistical analysis for correlation of K with the blood urea nitrogen, creatinine, and plasma 17-hydroxycorticosteroid levels by the method of least squares has been carried out in an attempt to define the factors responsible for the decreased action of insulin found in the uremic patients. Correlation could not be demonstrated between K and creatinine or 17-hydroxycorticosteroids. When K was plotted against blood urea nitrogen for the entire uremic group, r equaled only −.29. However, separation of the data for uremic patients by age-group showed that there was fair correlation between K and blood urea nitrogen in the young patients ($r = -.63$) with no correlation in the old patients ($r = -.029$). In the limited amount of data available, there was no apparent correlation between the rate of fall of blood glucose

TABLE 2

K values in normal subjects, uremic patients, and age comparable controls of Silverstone et al.[15]

Test Used	Subject Group	Number of Subjects	K (% Fall/Min.) Average	σ*	$\frac{1}{\sqrt{M^*}}$
Intravenous Glucose Tolerance Test	Normals Age 15-22	7	1.83	0.61	0.231
	Normals (15) Under 37	12	1.68		
	Normals Age 29-43	4	2.05	0.168	0.063
	Normals (15) Age 37-58	11	1.44		
	Uremics Under 60	4	0.83	0.429	0.162
	Normals (15) Over age 58	12	0.98		
	Uremics Over age 60	3	0.77	0.38	0.143
Glucose-Insulin Tolerance Test	Normals Under 35	8	5.18	1.02	0.385
	Normals (15) Under 37	12	6.39		
	Normals (15) Age 37-58	11	3.61		
	Uremics Under 60	7	1.47	0.50	0.188
	Normals (15) Over age 58	12	2.49		
	Uremics Over age 60	8	1.39	0.315	0.119

*$\frac{\Sigma(x-\bar{x})^2}{N} = \sigma$ = S.D.; $\sqrt{M} = \frac{\sigma}{\sqrt{N}}$ = s.e.m = $\sqrt{M}$

in the glucose-insulin tolerance test and serum sodium or potassium levels. In figure 4, the data for K vs. urea nitrogen in uremics under sixty years of age are shown, together with the calculated line of best fit. The equation for this regression line is $y = 2.96 - 0.0146X$. The standard error of the estimates of the points on the regression line $(Sy)^*$ is $\pm$ 0.40. Although the correlation is suggestive, the small number of cases available for analysis makes further interpretation unjustified.

5. *Dog Glucose Tolerance Tests.* In figures 5 and 6, representative intravenous glucose tolerance tests in normal dogs with and without the prior injection of urea, 2 gm. per kilogram body weight, are shown. Three of the five tests performed after urea and glucose showed greater initial hyperglycemia than after glucose alone.

* $Sy = \sigma y \sqrt{1 - r^2}$

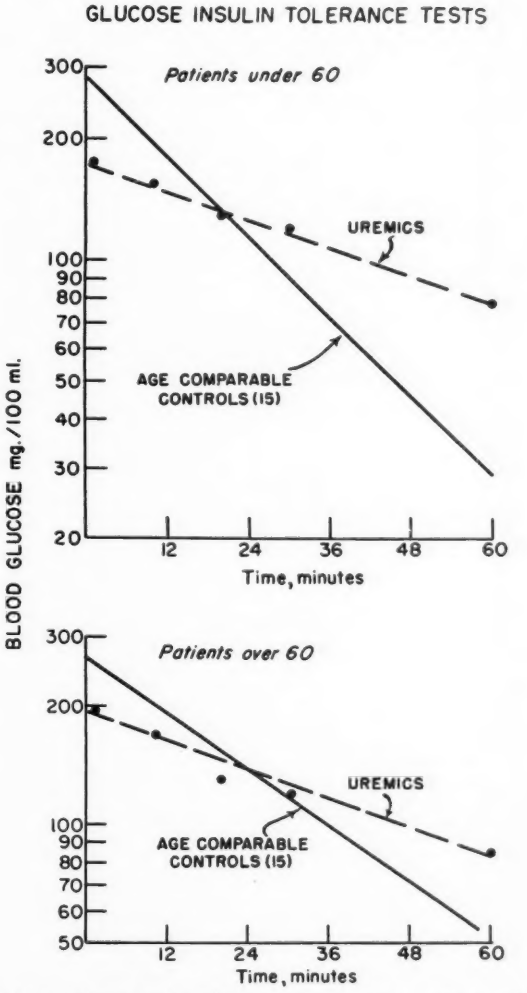

FIG. 3. Glucose-insulin tolerance tests in uremic patients and age comparable controls.[15] Zero time is the time of insulin administration and coincides with cessation of the glucose infusion.

In two of these, the glucose disappearance rate was slower than in the control test. In one other, initial hyperglycemia and glucose disappearance rate were the same after urea and glucose as in the control test. In the fifth dog, the initial hyperglycemia was greater after urea and glucose, but the glucose disappearance rate was more rapid than in the control test. The K values for these tolerance tests are listed in table 3. The same experiments, performed with creatinine, 2.5 gm. intravenously, instead of urea showed no differences from the control values.

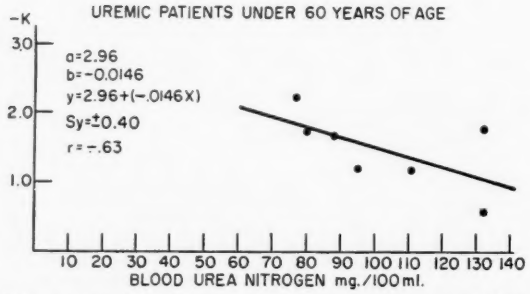

FIG. 4. Correlation between blood urea nitrogen and K (per cent fall per minute) in the glucose-insulin tolerance tests in uremics under sixty.

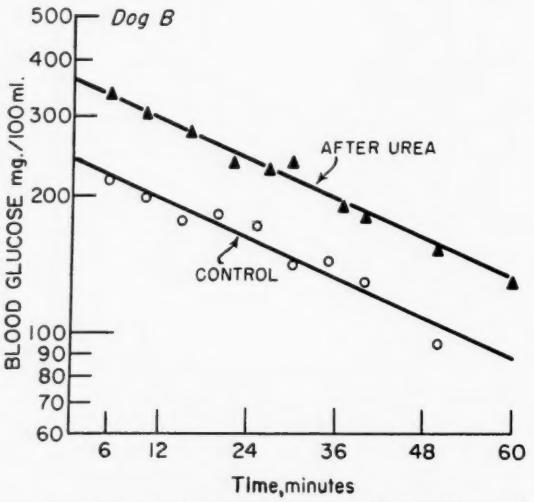

FIG. 5. Intravenous glucose tolerance test in Dog B before and after the administration of intravenous urea.

One possible explanation for the variable effects obtained in the dogs given urea could be an increase of the osmotic pressure of the blood in these animals. For this reason, these experiments were repeated with isosmotic doses of sodium chloride, 1.0 gm. per kilogram body weight.

Four of the five tests showed slight hyperglycemia after sodium chloride injection. The K values, calculated from the average blood glucose values in control and test animals, were: control, 2.35 per cent per minute; after 1 gm. sodium chloride per kilogram body weight, 1.68 per cent per minute.

6. *Effects of Oral Urea on Glucose Tolerance in Three Normal Subjects.* Standard intravenous glucose tolerance tests were performed on three normal male medical students, ages twenty-four to thirty-four, before and after the oral ingestion of 60 gm. urea in four divided

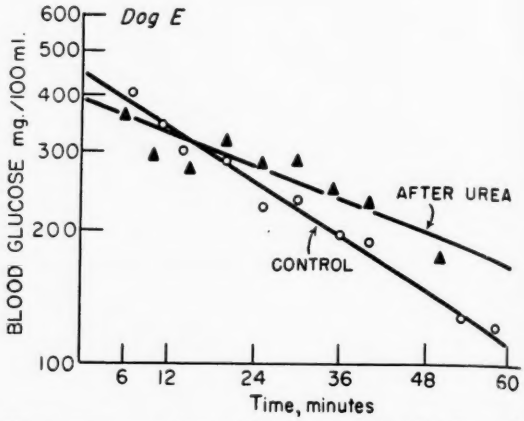

FIG. 6. Intravenous glucose tolerance test in Dog E before and after the administration of intravenous urea.

TABLE 3

Glucose tolerance tests in dogs*

Subject	K, % Fall/Minute Control	After urea
Dog A	3.15	3.13
Dog B	1.70	1.72
Dog C	2.70	4.10
Dog D	2.40	2.00
Dog E	3.35	1.36
		After sodium chloride
Five Dogs†	2.35	1.68

*1 gm./kg. body weight, with and without urea, 2 gm./kg. body weight.

†0.5 gm./kg. body weight, with and without 1 gm. sodium chloride/kg. body weight.

doses per day for three and one-fourth days. In two subjects, the blood urea nitrogen was moderately elevated (41 and 35 mg. per 100 ml. on the morning of the second glucose tolerance test, peak value during the previous days 41 and 41 mg. per 100 ml., respectively). The third subject had a peak blood urea nitrogen of 28 mg. per 100 ml. with a blood urea nitrogen of 21 mg. per 100 ml. on the morning of the second test. In each subject, the K value was lower after the ingestion of urea than it had been before urea ingestion. The K values are listed in table 4 and the curves from subject AG are shown in figure 7.

7. *In vitro Studies.*

a. *Glucose Uptake by Rat Diaphragm.* In six experiments, glucose uptake by rat diaphragm without added insulin averaged 0.36 mg. per 100 mg. tissue; with insulin, this increased to 0.69 mg. per 100 mg. tissue (three experiments). Incubations carried out with insulin plus urea gave a value of 0.76 mg. per 100 mg.

TABLE 4

Glucose tolerance tests in three normal subjects*

Subject	Specimen	BUN, mg./100 ml.	K, % Fall/Minute
AG	Control	12.5	5.50
	After urea	41	3.50
AN	Control	17.5	2.24
	After urea	21	1.78
RM	Control	9.5	5.30
	After urea	35	4.80

*0.5 gm. glucose intravenously per kg. body weight before and after 60 gm. urea orally for three and one-fourth days.

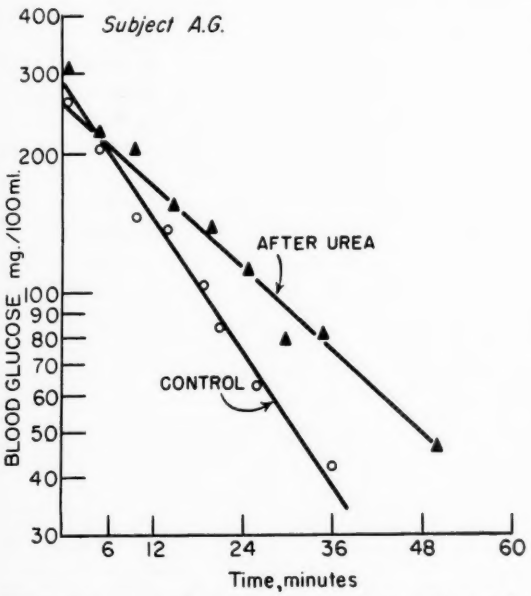

FIG. 7. Intravenous glucose tolerance tests in subject A.G. before and after urea ingestion.

tissue (three experiments). This figure, which is similar to that obtained without urea, shows that urea, in the concentrations used, had no demonstrable effect on the action of insulin in this system.

b. *Insulinase Assay.* Neither urea nor creatinine had any effect on the release of I^{131} from labeled insulin by rat liver extract. In control samples, prepared by heating the liver extract to 80° C. for ninety minutes, 30 per cent of the iodine was liberated. Active extract alone gave 65 per cent release (eight experiments); with added urea (five experiments), 64 per cent release; with added creatinine (three experiments), 63 per cent release. Thus, these compounds, under the circumstances of this study, had no influence on this enzyme system.

DISCUSSION

The data obtained in this study confirm the studies made many years ago[4,5,6] which report decreased glucose tolerance in patients with uremia. The evidence presented here for the presence of decreased action of insulin in uremic patients seems convincing, and helps to clarify the mechanism of the altered glucose tolerance tests. The reason for the change in insulin activity is less clear.

Diminished effect of exogenous insulin has been reported in various clinical disorders that seem widely separated from the classic disorders of carbohydrate metabolism. Olsen and Neutzel[19] gave one-fortieth unit regular insulin per kilogram of body weight to patients with various chronic illnesses, including benign and malignant essential hypertension, and found decreased effect of insulin in a variety of clinical circumstances in a degree that correlated roughly with the severity of the illness. In six patients with nephritis, this phenomenon was present only during the active phase of their renal disease. No uremic patients were studied. These authors postulated that the "adrenal response to stress" was the explanation for their results. Burns et al.[20] found that oral glucose tolerance tests were abnormal in acutely ill patients and that these abnormalities could be aggravated by cortisone administration. Standard insulin tolerance tests (0.1 unit regular insulin per kilogram body weight intravenously) gave normal results in these cases but became abnormal during cortisone therapy. They suggested that these changes were due to some interaction between the adrenal hormone and stress, although they did not think that adrenal hyperactivity per se accounted for the observed abnormalities in carbohydrate metabolism. It is worthy of note that the fasting blood sugars in the patients in both of these studies were normal. The elevated fasting blood sugars in our patients suggest that factors other than those operative in the studies of Olsen and Neutzel and of Burns et al. may be responsible for the changes in uremia.

The plasma 17-hydroxycorticosteroids (table 1) in the uremic patients reported here ranged from definitely normal levels (12 γ per 100 ml.) to moderately elevated levels in some cases (30 γ per 100 ml.). No correlation was found between these values and the degree of insulin action and, indeed, in the patient with the most marked changes, the plasma level of 17-hydroxycorticosteroids was normal (14 γ per 100 ml.). This makes it unlikely that increases in the plasma levels of adrenal steroids are a primary factor in the production of the reduced insulin effect, although they may play an accessory role.

That urea itself may have a specific influence on glu-

cose metabolism has not been suggested previously. The suggestive correlation between decreased insulin action and blood urea nitrogen levels in the young group of uremic patients, the alterations produced in the glucose tolerance curves in normal human subjects and in dogs following artificial elevation of the blood urea nitrogen to pathologic levels, and failure to obtain similar effects with creatinine are in accord with this concept. On the other hand, administration of sodium chloride to dogs also resulted in modification of the glucose tolerance test similar to that found with urea. The fact that sodium chloride remains in the extracellular fluid suggests that, if anything, the osmotic effects of sodium chloride are greater than those of equivalent loads of urea, which rapidly equilibrate with total body water. In addition, in the normal subjects given urea, the blood urea nitrogen levels were elevated for three days, and little if any acute osmotic effects can be implicated in subjects taking oral urea over this period of time. Thus, although an osmotic effect may partially explain the effects of urea, a specific intracellular effect of urea itself might still be present both in our dogs and in uremic patients. In the absence of the availability of a nontoxic substance which is distributed completely in total body water and which could be administered in the same dose as urea, it is not possible to test this hypothesis definitively.

Our studies of the effect of urea on glucose uptake by rat diaphragm and on insulinase activity of rat liver extracts do not clarify the mechanism by which urea might exert such an effect. It has been reported that urea, in concentrations similar to those observed in the serum of patients with uremia, can cause reversible denaturation of various enzymes and can produce decreased activity of certain enzyme systems.[21,22] The correlation of these effects with the concentration of urea was observed to follow a hyperbolic curve; therefore, relatively low concentrations of urea result in moderate decreases in enzyme activity. Such effects have not been demonstrated with any of the enzymes concerned with glucose metabolism. However, it should be noted that careful studies of purified preparations of these enzymes have not been reported.

It seems likely that many factors are involved in the changes in glucose metabolism seen in uremia. Nonspecific factors related to food intake, nausea and vomiting and moderate elevation of the plasma 17-hydroxycorticosteroids may be responsible in some patients. In addition, a specific effect of urea and/or some other substance which accumulates in the blood in uremia may play a role.

Since the studies reported here were performed in nondiabetic patients, it is not possible to draw direct conclusions from these results in relation to the course of diabetes mellitus in uremia. It would be desirable to be able to carry out these studies in diabetic patients, but the formidable difficulties inherent in any attempt to compare glucose tolerance data from different diabetic patients are well known.[23] Preliminary studies in three diabetic patients with uremia, carried out in this laboratory, gave results similar to those reported here but critical interpretation of the data was impossible because of variation in the severity and degree of control of the diabetes.

In spite of this, when the data obtained in nondiabetic uremic patients are considered together with the clinical observations that the course of diabetes during uremia bears little relationship to the renal lesion found in these patients, it seems unlikely that a specific renal lesion associated with uremia could influence favorably the course of diabetes mellitus. Indeed, unless care is taken in assessing the clinical status of uremic patients, an incorrect diagnosis of mild diabetes with intercapillary glomerulosclerosis could be made from the fasting blood sugar and the results of a glucose tolerance test in a patient with renal insufficiency.

SUMMARY

1. Oral and intravenous glucose tolerance tests in nondiabetic uremic patients showed abnormalities similar to those seen in mild diabetes mellitus.

2. Glucose-insulin tolerance tests indicated the effect of insulin is decreased in uremia.

3. The degree of decreased insulin action found in young uremic patients correlated roughly with the level of blood urea nitrogen. This was not true in elderly individuals.

4. The administration of urea intravenously to normal dogs resulted in the inconstant production of diabetic-type intravenous glucose tolerance curves in these dogs. Isosmotic doses of sodium chloride resulted in changes in glucose tolerance in dogs similar to those seen when urea was given.

5. The oral ingestion of urea by three normal subjects for three and one-fourth days was associated with a decreased rate of glucose disappearance from the blood in these individuals.

6. Urea did not influence rat liver insulinase activity or the uptake of glucose by rat diaphragm in vitro.

7. It is suggested that urea may have a specific effect on glucose metabolism in uremia. The mechanism of this effect is unknown.

8. These data do not support the thesis that the course

of diabetes mellitus is favorably influenced by the onset of intercapillary glomerulosclerosis with uremia.

SUMMARIO IN INTERLINGUA

Un Studio Del Mechanismo Del Tolerantia Anormal Pro Glucosa, In Uremia E Hyperazotemia Experimental

1. Tests del tolerantia pro glucosa oral e intravenose in non-diabetic patientes uremic revelava anormalitates simile a illos vidite in leve formas de diabete mellite.

2. Tests del tolerantia pro glucosa-insulina indicava que le effecto de insulina es reducite in uremia.

3. In juvene patientes con uremia, le grado del reduction in le action de insulina se monstrava grossiermente correlationate con le nivello de nitrogeno in le urea del sanguine. In patientes de etates plus avantiate, le correlation mentionate non esseva constatate.

4. Le administration intravenose de urea a canes normal resultava in le formation inconstante del typo diabetic de curvas de tolerantia pro glucosa intravenose. Doses iso-osmotic de chloruro de natrium resultava in le canes in alterationes del tolerantia pro glucosa, simile a illos vidite post le administration de urea.

5. Le ingestion oral de urea per tres subjectos normal durante un periodo de tres dies e un quarto esseva associate con un relentation del disparition de glucosa ab le sanguine de iste individuos.

6. Urea non influentiava le activitate de insulinase in hepate de ratto o le acceptation de glucosa per diaphragma de ratto in vitro.

7. Es suggerite que urea ha possibilemente un effecto specific super le metabolismo de glucosa in casos de uremia. Le mechanismo de iste effecto non es cognoscite.

8. Iste datos non supporta le these que le curso de diabete mellite es influentiate favorabilemente per le declaration de glomerulosclerosis intercapillar associate con uremia.

ACKNOWLEDGMENT

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Peripheral Vascular Complications in Diabetes Mellitus

A Survey of 3,600 Cases

David W. Kramer, M.D., and Paul K. Perilstein, M.D., Philadelphia

The association of diabetes with pathologic changes in the arteries is indisputable.[1] Whereas many authors have implied or definitely stated that diabetes is in some way responsible, others[2] have challenged this relationship. The latter group has questioned whether it was necessary to maintain normal levels of blood sugar and aglycosuria if the patient's general condition was satisfactory. There also has been a tendency to attribute the development of complications either to the severity of the diabetes or to the duration of this disease. With these thoughts in mind, we are offering an analysis of a large group of diabetics. Three thousand and six hundred patients have been observed by the authors since 1921. The first series of 1,000 cases was gathered between 1921 and 1930; the second series of 1,000 cases, from 1931 to 1941; series III, 1,000 cases, from 1942 to 1951; and the present series IV, 600 cases, from 1952 to 1956 inclusive. They were consecutive cases seen in office and in the hospital. However, the vast majority were office cases. It may be added that the entire group has been observed since the advent of insulin; patients ranged from potential cases of diabetes to severe types and were of all ages.

In this survey, our interest was focused on the peripheral vascular system. Patients were questioned about symptoms of the circulation, and this was followed by examination of the extremities for evidence of impaired circulation.[3] Patients who showed possible involvement of the arteries were further examined by circulatory function tests, such as the oscillometer, capillary response to histamine, plantar ischemia and venous filling time. When necessary, skin surface temperature studies were performed, and, when indicated, soft tissue X rays of the vessels were made.

Presented at the Seventeenth Annual Meeting of the American Diabetes Association in New York City on June 2, 1957.

*From the Vascular Clinic of Jefferson Medical College Hospital and Metabolic and Vascular Clinics of St. Luke's and Children's Medical Center, Philadelphia, Pennsylvania.

The cases were placed in three categories: (a) impaired circulation, (b) threatened gangrene and (c) gangrene. Evidence of impaired circulation consisted of symptoms such as claudication, coldness and numbness, diminished or absent pedal pulses, and subnormal circulatory function tests. Threatened gangrene was based on the presence of cellulitis with discoloration of the toes or ulcerations. Almost invariably, these patients had evidence of impaired circulation. Gangrene was applied to that group showing focal or extensive necrosis.

An analysis of the 3,600 cases has been arranged in table 1. A glance at the number of patients with involvement of the peripheral vascular system showed a steady rise from 17.3 per cent in the first series to 21.6 per cent in the second series, 50.7 per cent in the third and 58.6 per cent in the current series. A breakdown of these figures according to the degree of involvement showed that those patients who were listed in Group A, namely those with impaired circulation, showed a progressive rise from 8.8 per cent in the first series to 47.3 per cent in the fourth series. The threatened gangrene group similarly showed a rise in the incidence, from 2.8 per cent to 7.6 per cent in the third, and then dropped to 4.5 per cent in the fourth series. The obvious gangrene cases have been more or less consistent, ranging from 5.7 per cent in the first group to 7.2 per cent in the second and to 6.8 per cent in the fourth series.

In an attempt to obtain more information about the factors in the relationship of diabetes to peripheral vascular disease, the 1,000 cases in series III and the 600 cases in series IV were further examined according to duration of the diabetes, severity, age and sex. The findings were arranged in tabular forms.

Duration of diabetes has been mentioned as a possible influence in the development of complications.[4] This is particularly true in juveniles who show a very high incidence of pathologic changes in the vessels, especially the smaller arteries, after fifteen to twenty years of the disease.[5] However, in adults the duration of the diabetes cannot be an important factor when considering the

TABLE 1

Showing the incidence of vascular complications in 3,600 cases of diabetes

Type of Case	Series I 1921-1930 (1,000 cases)		Series II 1931-1941 (1,000 cases)		Series III 1942-1951 (1,000 cases)		Series IV 1952-1956 (600 cases)	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
a. Impaired circulation	88	8.8	108	10.8	372	37.2	284	47.3
b. Threatened gangrene	28	2.8	36	3.6	76	7.6	27	4.5
c. Gangrene	57	5.7	72	7.2	59	5.9	41	6.8
Total	173	17.3	216	21.6	507	50.7	352	58.6

incidence of vascular complications. It is not unusual to see patients with thrombosis or gangrene in whom the diabetes has never been recognized until the patient comes under observation for vascular disease. One cannot help being impressed with the occurrence of 129 cases in which diabetes was discovered for the first time. In addition, there were 102 cases in which the vascular disturbance was recognized in diabetes existing for less than a year. The highest incidence of complications of the peripheral arterial system was in patients with diabetes of one to five years' duration, 314 cases. These, added to the first two categories, "Recently discovered" and "Less than one year," indicate that more than 60 per cent had developed changes of the arterial system within the first five years of the diabetes. The next highest, 165 cases, occurred in the six- to ten-year group.

TABLE 2

Duration of the diabetes when the vascular condition was recognized (1,600 cases*)

Type of Case	Recently discovered	Less than 1 year	1 to 5 years	6 to 10 years	11 to 15 years	16 to 20 years	21 years plus	Not recorded
Impaired circulation	112	75	248	128	44	37	12	
Threatened gangrene	7	15	34	20	12	7	7	1
Gangrene	10	12	32	17	14	9	6	
Total	129	102	314	165	70	53	25	1

*Series III and IV.

The question may be raised as to whether or not the diabetic condition may have existed for some time before being recognized clinically, a question which cannot be taken up here.

Severity of the diabetes: It is difficult to find a method of grading diabetes according to severity. However, the following types seem to be commonly accepted: *mild* cases, those who can be controlled by diet alone or in whom the daily insulin requirement is 20 units or less. If the dose of insulin was as much as 40 units, patients were classed as *moderate* and when the daily requirement was more than 40 units, the classification was *severe.*

In table 3, it will be noted that of the 1,600 cases, 859 patients showed various degrees of vascular involvement. The highest incidence of vascular disturbances was noted in 539 patients with mild diabetes, with 216 cases occurring in the moderate diabetics and 99 cases in those who were classed as severe. It may be mentioned that gangrene was more frequent in severe diabetes (26.1 per cent) as compared to 8.3 per cent in the mild form.

TABLE 3

Various grades of vascular disorders according to the severity of the diabetes (1,600 cases*)

Type of case	Mild	Moderate	Severe	Not recorded
Impaired circulation	444	146	65	2
Threatened gangrene	50	33	17	2
Gangrene	45	37	17	1
Total	539	216	99	5

*Series III and IV.

In view of these figures, one would expect a high incidence of vascular disorders in the severe diabetic. However, the incidence of vascular disturbances among mild cases would indicate that there may be some other influences which are responsible for these findings.

Age: In table 4, analysis of the age groups was made in relation to the various degrees of peripheral vascular disorders. It was noted that the highest number of 348 cases of vascular disorders occurred in the seventh decade. The next highest was 296 in the sixth decade group. The occurrence of peripheral vascular disorders in the younger age groups is quite low. Six cases were found among juvenile diabetics in the second and third decades. This concurs with White's observation of the infrequency of these complications in juvenile diabetics, less than ten years' duration.

Sex: A survey of the 1,600 cases showed that the male-

TABLE 4

Age at time vascular disorder was detected (1,600 cases*)

Type of Case	Number of Patients at Age (Years)							
	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80 plus
Impaired circulation	2	3	16	90	221	267	52	5
Threatened gangrene	—	1	2	11	41	38	10	—
Gangrene	—	—	—	10	34	43	12	1
Total	2	4	18	111	296	348	74	6

*Series III and IV.

female ratio in the entire group was 1:1.32. A further analysis of the various degrees of vascular disorders in relation to sex is recorded in table 5. It will be noted that there were 475 cases with vascular disorders among the females and 384 among the males. This preponderance, although slight, is evidenced in all three of the grades, namely impaired circulation, threatened gangrene and gangrene. The ratio of the degree of involvement in reference to male and female was 1:1.24. It may be pertinent to mention that most of these cases were in the older age groups, when the diabetic female has reached the menopausal stage and no longer benefits from the protective influence of the estrogens.

TABLE 5

Relative incidence of vascular disorders in reference to sex (1,600 cases*)

Type of Case	Males	Females	Ratio
Impaired circulation	294	363	1:1.23
Threatened gangrene	46	56	1:1.22
Gangrene	44	56	1:1.27
Total	384	475	1:1.24

*Series III and IV.

DISCUSSION

The problem of the relationship of diabetes mellitus and the high incidence of peripheral vascular complications is not new. Hitherto, two factors that have been considered important in the development of these complications have been the duration and/or the severity of the diabetes. The result of this survey showed that there was a progressive increase in the incidence of vascular changes, ranging from 17.3 per cent in the first series of 1,000 cases to 58.6 per cent in the current series. When taking these figures into consideration the fact that diabetes is being detected more often in early stages and that constant efforts are being made to control possible hypercholesterolemia by low-fat diet and other therapeutic means must be remembered. The apparent increase of the incidence of vascular pathology is striking and poses the question as to whether we are justified in assuming that the diabetes per se is responsible for these changes. The tables show that the high incidence of peripheral vascular complications occurred in diabetics in whom the condition was recognized within the first five years of the disease. Similarly, the high incidence of peripheral vascular disturbances was more significant in the milder forms of diabetes. The findings of the survey suggest that the basic cause for atherosclerosis might best be sought for in other fields. Further investigations in the field of metabolic disorders, besides cholesterol and carbohydrates, may give added information. Observation of uric acid levels in a series of patients with vascular diseases has indicated that it is not unusual to find hyperuricemia.[6] This need not necessarily imply that uric acid itself is responsible for these changes, but a disturbance of the uric acid metabolism may be a contributory influence. In delving into the possible explanation for atherogenesis, a greater knowledge of tissue metabolism of the individual cells may be helpful.[7] With a better understanding of the functioning of the intimal cells and what influences may disturb normal cell metabolism, the answers to these problems would be more easily achieved.[8]

Thus far, we have been successful in developing ways and means of detecting an impaired circulation even in the early stages. By recognizing these cases and treating them accordingly, it has been possible to contain the incidence of gangrene. Since it has been demonstrated that some form of atherosclerosis may be reversible, efforts should be made to investigate the various causes that induce atherosclerosis.

SUMMARY AND CONCLUSIONS

1. A survey of 3,600 cases of diabetes has indicated that the incidence or diagnosis of vascular complications is increasing.

2. The duration of diabetes alone is not a significant factor. The highest incidence seems to be in the first five years. This applies to the adult groups of diabetics.

3. Similarly, the severity of the diabetes is not a definite factor, because the vast majority of complications have occurred in patients with milder forms of the disease.

4. Age and sex have, likewise, been analyzed and discussed, and do not offer any significant explanation for the rise of the incidence of vascular complications.

5. Although our findings lead us to assume that diabetes per se is not responsible for the increasing incidence of complications, we still believe that it is advisable to continue with our rigid control of the diabetes and also with low-fat diets.

SUMMARIO IN INTERLINGUA

Complicationes Periphero-Vascular In Diabete Mellite: Un Revista De 3.600 Casos

1. Un revista de 3.600 casos de diabete ha indicate que le incidentia del diagnose de complicationes vascular se trova crescente.

2. Le duration del diabete non es—per se—un factor significative. Le plus alte incidentia pare concentrar se in le prime cinque annos del diabete. Isto vale pro le patientes de etate adulte.

3. Similemente, le grado de severitate del diabete non es definitemente un factor, proque le vaste majoritate del complicationes esseva constatate in patientes con plus leve formas del maladia.

4. Etate e sexo ha etiam essite analysate e discutite e etiam non offere un explication significative pro le augmentate incidentia del complicationes vascular.

5. Ben que nostre constatationes fortia nos a concluder que diabete per se non es responsabile pro le crescente incidentia de complicationes, nos adhere—non-obstante—al conviction que il remane recommendabile continuar le rigide control de diabete e etiam le dieta a basse contento de grassia.

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The real frontier areas of biochemistry now reach to the borderlines of physiology, genetics, cytology, medicine and theoretical chemistry. The biochemical description of muscular contraction, nerve conduction, glomerular filtration, and secretion and membrane phenomena is still relatively virgin territory. The structural analysis and interpretation of the way in which mitochondria, nuclei, membranes, myelin sheaths and other complex cellular structures are constructed in a chemical sense have yet to reach even the blueprint stage. A fabulous area of exploitation awaits the investigators who can hurdle the conceptual barriers in the way of the biochemical study of hormone action at the molecular level. If current progress on the reconstruction of in vitro systems or synthesis of protein is to serve as a guide, then we must surely anticipate the polygamous marriage in the not too distant future, of biochemistry with genetics, immunology, hematology and virology. The older problems of biochemistry are clearly moribund but the newer problems have the fascination and challenge of youth.

DeWitt Stetten, Jr., p. 9, in *Currents in Biochemical Research*, edited by David E. Green.

Tolbutamide and the Onset of Alloxan Diabetes in Rats

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It has been established that certain sulfonylureas are effective in lowering the blood sugar both in man and animals. However, the mechanism by which hypoglycemia is produced has not been clarified. One of the most favored hypotheses states that the sulfonylureas cause hypoglycemia by stimulating the beta cells of the pancreas to release more insulin. One of the principal pieces of evidence in support of this hypothesis is the fact that most of the sulfonylureas have no effect either on severely alloxan diabetic[1] or depancreatized animals,[2] i.e., they are ineffective in animals without functioning beta cells. The presence of even a small amount of functioning islet tissue is associated with the hypoglycemic response to these sulfonylureas.[3] Cross-circulation experiments in which one of the sulfonylureas was given to the "normal" donor dog, with a consequent reduction in blood sugar of the recipient depancreatized dog,[4] lend added support to this view. Also, direct perfusion of small amounts of tolbutamide into an artery supplying the pancreas of dogs suggests that resultant hypoglycemia is brought about by direct action on islet tissue.[5]

Recently, Volk and Lazarus[6] reported that intravenous administration of tolbutamide to rabbits daily for seven days resulted in a complete degranulation of the beta cells in some of the animals studied. They interpret this to mean an increase in the release of insulin with either normal or possibly increased insulin production.

The present study was concerned with the further investigation of the hypothesis that beta cell stimulation is the mode of action of tolbutamide* in the rat. Two steps were projected: (1) a regimen of tolbutamide treatment that would bring about beta cell degranulation, and (2) if such a regimen were found, a functional test was to be applied (the triphasic response to alloxan) which might be affected by partial or complete depletion of the animal's insulin stores.

*Orinase, kindly supplied by The Upjohn Company.

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METHODS

The following procedure led to a regimen for producing histological evidence of degranulation. Male albino rats weighing between 150-200 gm. were given 100 mg./kg. of tolbutamide by stomach tube repeatedly on various time schedules. They were then sacrificed, the pancreas fixed in Bouin's solution, and the tissue stained for islet cell differentiation, using a modification of the aldehyde-fuchsin technic.[7]

The procedure selected for detecting depletion of insulin stores was to follow the blood sugar of tolbutamide-treated animals after a diabetogenic dose of alloxan monohydrate. Alloxan monohydrate, when given in an appropriate dose, causes selective necrosis of the beta cells of the pancreatic islets. Following injection of the toxic agent, the blood sugar of the animals exhibits a triphasic response which consists of an initial hyperglycemia followed by a transient hypoglycemia terminating in a permanent hyperglycemia.[8] The initial hyperglycemia is attributed to stimulation of the adrenosympathetic system and usually occurs within the first twelve hours. The hypoglycemic phase usually occurs within twenty-four hours and is attributed to the liberation of preformed insulin from the damaged beta cells. Permanent hyperglycemia occurs within forty-eight hours and is due to the failure of the damaged beta cells to produce insulin. The part of this triphasic response to alloxan which was of particular interest in this study was the second or hypoglycemic phase. If treatment of the animals with tolbutamide resulted in degranulation of the beta cells, and if this degranulation were synonymous with either a decrease or depletion in insulin stores, then the hypoglycemic phase either should be diminished or eliminated because there would be little or no preformed insulin liberated from the damaged beta cells.

This line of reasoning was tested using the following experimental procedure:

Male albino rats weighing 200-300 gm. were divided into four groups:

The first group was given tolbutamide by gavage, 100 mg./kg. three times per day (t.i.d.) for five days. At the time of the last dose of tolbutamide, these animals were also given a subcutaneous injection of 125 mg./kg. of alloxan.

The second group was treated with tolbutamide for five days in the same manner as the first group but at the time of the last dose of tolbutamide they were given a subcutaneous injection of water.

The third group of animals was given water by gavage, 1.5 ml. three times a day for five days, and at the time of the last dose they were given a subcutaneous injection of 125 mg./kg. of alloxan.

The fourth group of animals was handled like the third group except that at the time of the last dose of water they were given a subcutaneous injection of water.

All animals were fasted twenty-four hours prior to the subcutaneous injection of the alloxan or the corresponding water control so that fasting blood sugar samples could be taken at the time of this injection. Blood samples from a lateral tail vein were taken at this time, at every hour for the first eight hours, and at every two hours for the next sixteen hours; a final sample was taken at forty-eight hours. Blood sugar was determined by the Nelson-Somogyi method.[9]

RESULTS

Figure 1 is a photomicrograph of the pancreatic tissue of an untreated rat. The darkly staining beta cells occupy the center of the islet and show the normal arrangement of granules (black) oriented toward one pole of the cell. When six animals were given a single dose of tolbutamide and biopsies of their pancreatic tissue studied, no alteration in this normal histological picture could be detected. Treatment with tolbutamide three times per day for one day also failed to produce any demonstrable alteration in the normal appearance of the islet tissue. However, the beta cells of islet tissue removed from most animals treated with tolbutamide three times per day for three days showed marked degranulation (see figure 2). This histological picture was found in five of the six animals examined in this series. When animals were pretreated with tolbutamide three times per day for five days, four of the six in this series had almost complete degranulation of beta cells (see figure 3).

The results of experiments involving the triphasic blood sugar response to alloxan injection are shown in figure 4. The open triangles represent the mean values for four animals which were given water by gavage t.i.d. for five days and at the time of the last such gavage (time "0") they were given a subcutaneous injection of

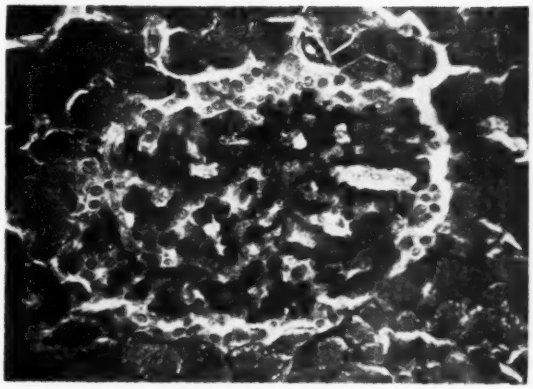


FIG. 1. Pancreatic islet of untreated rat, showing the orientation of granules (black) toward one pole of the beta cells. Aldehyde-fuchsin stain. X500.

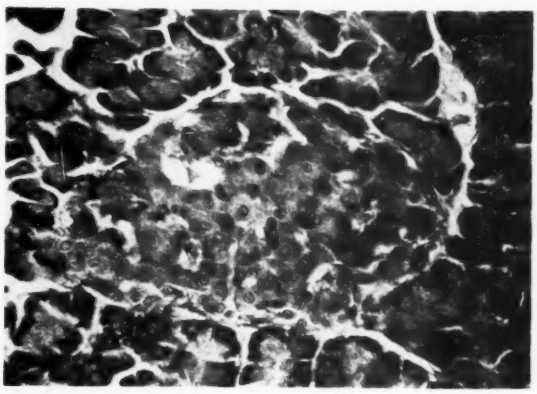


FIG. 2. Pancreatic islet of rat treated with tolbutamide for three days showing marked degranulation of the beta cells. Aldehyde-fuchsin stain. X500.

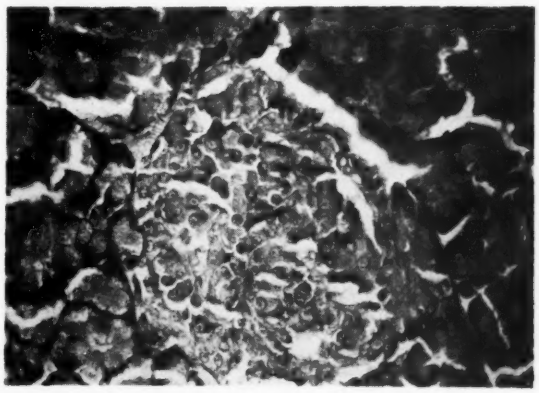
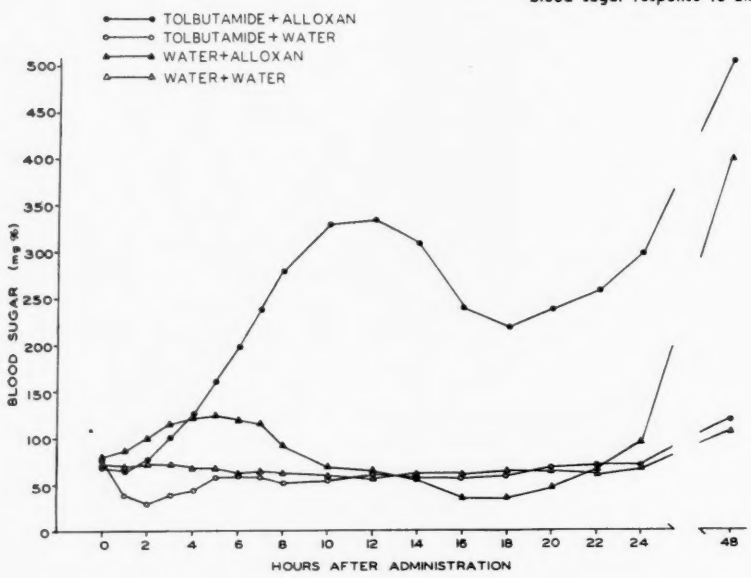


FIG. 3. Pancreatic islet of rat treated with tolbutamide for five days showing almost complete degranulation of the beta cells. Aldehyde-fuchsin stain. X500.

FIG. 4. The effects of tolbutamide pretreatment on the triphasic blood sugar response to alloxan injection.



water. The mean initial blood sugar was 75 mg. per cent. The blood sugar tended to decrease slightly for the first twelve hours, returned to normal at the end of twenty-four hours, and was slightly above normal at the end of forty-eight hours.

The open circles in figure 4 represent the mean values of four animals which were given tolbutamide t.i.d. for five days and at the time of the last tolbutamide gavage (time "0") they were also given a subcutaneous injection of water. The initial mean blood sugar was 77 mg. per cent. These animals showed a typical hypoglycemic response to the last dose of tolbutamide which persisted for four hours. Following this, the curve was essentially the same as that for those animals gavaged with water just described (open triangles).

The closed triangles in figure 4 represent the mean values for eight animals which were gavaged with water t.i.d. for five days and at time "0" were given a subcutaneous injection of alloxan. The initial mean blood sugar was 79 mg. per cent. The blood sugar of these animals exhibited the characteristic triphasic response to the injection of alloxan. The initial hyperglycemia was maximum within seven hours after which there was a decrease in blood sugar, sometimes to severely hypoglycemic levels. Then the blood sugar rose to severely hyperglycemic levels at forty-eight hours.

The closed circles in figure 4 represent the mean values of eight animals which had been gavaged with tolbutamide t.i.d. for five days and at time "0" were given a subcutaneous injection of alloxan. The initial mean blood sugar was 70 mg. per cent. These animals did not exhibit an hypoglycemic episode of as large magnitude as that seen in the other tolbutamide-treated animals (open circles). Hyperglycemia quickly appeared, reaching a maximum level in twelve hours, which was much more severe than that exhibited by the animals gavaged with water and then injected with alloxan (closed triangles). Although the mean values for the blood sugar of these eight animals have been plotted between hours twelve to twenty-four, this may be misleading if data for each individual animal are not examined in this time interval. Two of these animals did not show a fall in blood sugar during this time; two others showed a decrease to approximately 300 mg. per cent, and the other four animals showed decreases in blood sugar to levels ranging from 200 mg. per cent to 50 mg. per cent. Thus, in this period, the variation around the mean is not uniform and a definite trend toward absence of this phase (no hypoglycemic phase) is noted. At forty-eight hours all eight animals were severely hyperglycemic.

DISCUSSION

The present study adds support to the hypothesis that tolbutamide produces hypoglycemia by causing an increase in insulin secreted by the pancreas. The histolog-

ical changes in the pancreatic tissue of rats treated five days with thrice daily tolbutamide gavage agrees with the results which Volk and Lazarus observed in rabbits.[4] The data obtained from determinations of the blood sugar response in tolbutamide-pretreated animals after a diabetogenic dose of alloxan strongly suggest that the degranulation seen represented a decrease in the insulin stores in the pancreas. Thus, on inspection of the plotted data it is evident that pretreatment with tolbutamide alters the triphasic response to alloxan in two ways: 1. The tolbutamide-treated animals showed a more severe and prolonged initial hyperglycemia than was present in the controls. 2. The hypoglycemic phase did not reach the usual low levels since it was not found at all in some animals while appearing in variable degrees in others.

The first of these alterations could be explained on the basis of a reduction in the amount of insulin which the animals were able to mobilize in an effort to overcome the rise in blood sugar brought on by the adrenosympathetic response. It will be noted in figure 4 that these animals showed only a slight decrease in blood sugar one hour following treatment with tolbutamide and alloxan when compared with the large decrease in the animals treated with tolbutamide alone. This slight decrease can be taken as the algebraic sum of the hypoglycemic effect of the tolbutamide and the hyperglycemic effect of the alloxan. Soon the hyperglycemic effect of the alloxan apparently predominates and the rising blood sugar is relatively unchecked in this first phase.

The second of these alterations in the triphasic response could be explained on the basis of variations in the amount of insulin stores in the pancreases of the animals treated with tolbutamide. This could account for the variations in the degrees of hypoglycemia produced. From the histological study presented, it should be recalled that only four of the six animals treated with tolbutamide for five days showed nearly complete degranulation of the beta cells. Since it was not possible to examine histologically the beta cells of the animals before treatment with alloxan (in the functional study) it is impossible to state whether the level of blood sugar reached in the period from twelve to twenty-four hours was actually related to the degree of degranulation present before the alloxan injection.

SUMMARY

The hypoglycemic phase of the onset of alloxan diabetes is generally held to be due to the release of preformed insulin from necrotic beta cells in the pancreatic islets. Tolbutamide may induce hypoglycemia by forcing production and/or releasing insulin at the same site within the pancreas. If reduction of stored insulin to subnormal levels is an important feature of tolbutamide action, then pretreatment with this agent might lessen or even eliminate the hypoglycemic phase after diabetogenic dosage of alloxan. This possibility received experimental support when rats were pretreated with tolbutamide three times per day for five days and then were given a diabetogenic dose of alloxan at the time of the last dose of tolbutamide. Blood sugar determinations begun at this time and continued for forty-eight hours were compared with responses in appropriate controls. A striking tendency toward a lessened severity of the hypoglycemic phase on the way to the usual third phase of permanent diabetes was observed in the tolbutamide-pretreated animals. Upon histological examination another group of rats similarly pretreated but not alloxanized showed almost complete degranulation of the pancreatic beta cells.

SUMMARIO IN INTERLINGUA

Tolbutamido E Le Declaration De Diabete Alloxanogene In Rattos

Le phase hypoglycemic del declaration de diabete alloxanogene es generalmente considerate como un effecto del liberation de insulina preformate ab necrotic cellulas beta in le insulas del pancreas. Tolbutamido pote inducer hypoglycemia per fortiar le production e/o le liberation de insulina al mesme sito intra le pancreas. Si le reduction del reservas de insulina usque a nivellos subnormal es un aspecto importante del action de tolbutamido, il seque que un pre-tractamento con iste agente reduce o mesmo elimina le phase hypoglycemic que occurre normalmente post un dosage diabetogene de alloxano. Iste possibilitate trovava supporto experimental quando rattos esseva pre-tractate con tolbutamido tres vices per die durante cinque dies usque illos recipeva un dose diabetogene de alloxano al tempore del ultime dose de tolbutamido.

Determinationes del sucro sanguinee, comenciate a iste tempore e continuate durante quaranta-octo horas, esseva comparate con le valores observate in appropriate animales de controlo. Esseva observate, in le animales pre-tractate con tolbutamido, un frappante tendentia de disveloppar un minus sever phase hypoglycemic ante le advenimento del usual tertie phase de diabete permanente.

Le examine histologic de un altere gruppo de rattos, similemente pre-tractate con tolbutamido sed non recipiente alloxano, revelava un quasi complete disgranulation del cellulas beta in le pancreas.

ACKNOWLEDGMENT

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Blood Amino Acids, Glucose and Appetite

Recently Mellinkoff, Frankland, and Greipel (*J. Applied Physiol.* 9:85, 1956) have measured the effect of amino acid ingestion and its accompanying hypoglycemia upon the arteriovenous (a-v) blood glucose difference. After the usual night-time fasting period, sixteen normal volunteers drank 250 ml. of 10 per cent amino acids. Blood specimens were taken from the antecubital vein and from one finger tip during fasting, thirty minutes after the test meal, and at hourly intervals for a period of four hours. Just before each sample was taken the subject was asked if he were hungry and the responses were graded from minus 1 (nausea) to 0 (not hungry) through plus 4 (ravenous).

For comparison the above procedure was also followed in ten normal volunteers, but a solution of glucose was used instead of amino acids. Calculation of the correlation between changes in the serum amino acid concentration and appetite showed a diminution in appetite as serum amino nitrogen concentration rose, and a rise in appetite as the amino nitrogen concentration decreased. The venous blood sugar and amino nitrogen concentrations changed in opposite directions. When the a-v sugar changes were expressed as the difference between the individual's fasting a-v sugar difference and each subsequent a-v sugar difference, the appetite rose as the a-v sugar difference diminished and vice-versa. Following the ingestion of glucose there was a significant correlation between changes in appetite and changes in both the venous blood sugar and the a-v sugar difference. There was no correlation between changes in appetite and changes in amino nitrogen concentration, following the ingestion of glucose. Mellinkoff, Frankland and Greipel, however, do not claim that these experiments prove or disprove the a-v sugar hypothesis, but rather that the data indicate metabolic changes associated with appetite variations under specific circumstances. One mechanism for the depression of venous blood sugar after ingestion of amino acids is the possible uptake of glucose by the muscles, suggested by the fact that the venous blood sugar was generally lowest when the a-v sugar difference was greatest.

Further experiments are necessary to determine the mechanism by which amino acid ingestion depresses blood sugar concentration and appetite, and the role of the a-v sugar difference under these circumstances. For the present, the exact mechanism by which appetite is stimulated remains shrouded in mystery.

From *Nutrition Reviews,* Vol. 16, No. 1, pp. 8-9.

The Use of Simple Enzymatic Tests for Glucose in Detection of Diabetes

Evaluation by Means of a Modified Glucose Tolerance Test

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Paper strips impregnated with a glucose oxidase enzyme system are now well known in the detection of glucose in the urine. Whatever limitations they may have in the day-to-day management of known diabetes, their extreme simplicity suggests very great usefulness in the detection of unknown cases of diabetes.

However, two aspects of their use have caused some hesitation in this regard: 1. Since the enzyme tests are self-developing, the imperfectly informed lay person might make unwarranted and possibly harmful conclusions concerning his own condition without the benefit of professional medical advice. To be sure, in most detection campaigns final action in seeking medical advice is up to the individual lay person, regardless of the method of detection used. 2. The enzyme tests are very sensitive, detecting 100 mg. of glucose per 100 ml. or even lower concentrations, and there is a reasonable concern that an unusually large number of false positive tests may result. Undesirable effects of this sensitivity would include wasted effort on the part of the detection group and unnecessary alarm and anxiety on the part of some members of the public. It is also possible that either fading or late development of the color in the enzyme tests might affect the results unpredictably.

An opportunity to gain information regarding these questions was presented by the diabetes detection drive sponsored by the Twin Cities Diabetes Association of Minneapolis and St. Paul as part of National Diabetes Week, Nov. 11-17, 1956. A modified glucose tolerance test was used to interpret the results of the urine tests. Comparison was possible between the two enzyme tests and the Dreypak, a copper reduction method widely used in diabetes detection.[1]

The results indicate that some self-interpretation of the enzyme tests undoubtedly occurred, but that this probably did not affect materially the number of cases reported to the detection committee. The enzyme tests appeared to have the same over-all degrees of sensitivity and reliability as the Dreypak.

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METHODS

Format of the Tests: Tes-Tape and Clinistix are paper strips impregnated with a glucose oxidase-orthotolodine system so that a blue color develops in the strip in the presence of glucose and oxygen.[2,3] They differ mainly in that Tes-Tape comes as thin yellow paper and Clinistix as rather thick white paper. Testing units supplied by Eli Lilly and Company consisted of a strip of Tes-Tape stapled to a foil-lined paper wrapper folded loosely over it. Clinistix testing units were supplied by Ames Company, Inc., and consisted of a strip of Clinistix enclosed in a tightly sealed foil envelope attached to a printed card. Both units provided space for recording identifying information and also for recording in simple fashion the results of the test as done at home—that is, whether or not a color developed in the strip. The Tes-Tape unit provided a simple color chart for matching.

The Dreypak was devised by Dr. Norman Drey for the St. Louis Diabetes Association. It consists of a strip of filter paper impregnated with sodium fluoride as a preservative and attached to a broader strip of plastic and cardboard. The filter paper is dipped into a urine specimen and allowed to dry. At the detection center the strips require development by immersion for one minute in Benedict's qualitative reagent (boiling). Reducing substance is indicated by the appearance of a yellow or orange color in the filter paper strip.[1]

Fifty thousand testing units were made available to the public through drug stores in Minneapolis and St. Paul. Most of these consisted of a single Dreypak, Tes-Tape, or Clinistix unit. About 7,000 were double units consisting of a Dreypak and Tes-Tape unit stapled together. Appropriate directions were given for the four types of unit, suggesting that the urine sample to be tested be passed after a meal high in carbohydrate. The

tests were re-inserted in identical envelopes and were distributed to the druggists in random fashion. The public was invited to make use of the tests through displays furnished for the nation-wide detection drive by the American Diabetes Association; no mention was made of the comparison being made.

An approximate estimate of tests actually distributed was made by collecting and sorting tests left with the druggists after the detection week.

Interpretation of the Tests: Test strips were returned by mail to the detection center, where they were read two days to two weeks after mailing. Reading of the enzyme strips was done by physician and lay members of the diabetes association. The main question for which an answer was sought was this: Is the sensitivity of the enzyme tests so great that an excessive number of false positive results will be obtained in comparison with a standard detection method (the Dreypak)? For this reason it was felt necessary to rate even doubtful or borderline tests as positive. Any blue or green coloring in the test strip was rated as a positive test even if it amounted to only a slight streaking. Further, all tests which were rated as positive at home were counted as positives, even though the color had faded afterward. All doubtful tests were checked by one of the authors.

The results were therefore deliberately weighted to give a maximum number of false positive tests. The effects of self-interpretation, of late development and of fading could not be estimated individually but may be assumed to have had a bearing on the results as well.

All Dreypak tests were processed in Benedict's qualitative reagent according to the technic recommended by the American Diabetes Association—by one technologist who had no knowledge of the results of the enzyme tests on any of the subjects. The Dreypaks were interpreted according to the recommendations of Dr. Drey and the Association.

Follow-Up Glucose Tolerance Tests: Everyone submitting a urine test found to be positive was offered a modified glucose tolerance test without charge. This consisted of a venous blood sugar determination two hours after ingestion of 100 gm. of glucose in the fasting state; 50 gm. of glucose was the dose used for children under the age of twelve. The blood samples were collected in fluoride tubes which were refrigerated and analyzed within three weeks by Benedict's method.[4] Normal values in the fasting state for this method lie between 60 and 90 mg. per 100 ml. The blood sugar test was offered only to persons who stated they had no previous knowledge of diabetes.

RESULTS

The results will be presented in the form of five tables.

Acceptance by the Public, and Self-Interpretation: Table 1 gives some idea of public response in relation to the type of test used.

TABLE 1

Percentage returned of four urine test units offered to the public

	Approximate number of tests distributed (Taken from druggists)	Returned to Detection Center	
		Number	Per cent
Dreypak	16,000	6,088	38
Tes-Tape	16,000	3,000	19
Clinistix	11,000	1,790	16
Double test (Dreypak and Tes-Tape)	7,000	1,687	25
All tests	50,000	12,565	25

Many people undoubtedly knew that a blue or green color on the enzyme strip indicated the presence of sugar, though no mention of this fact was made in the testing unit. Presumably some negative tests were not mailed back. The Dreypak, which had to be mailed in for processing, showed a much higher percentage return.

The likelihood that a certain amount of self-reading of the enzyme tests occurred is also shown in table 2 by the percentage of positive results yielded by the various tests:

TABLE 2

Percentage positive of urine tests returned, according to the type of test used*

	Number of Positive Tests	Per cent Positive of Tests Returned	Approximate Per cent Positive of Tests Distributed
Dreypak	233	3.8	1.5
Tes-Tape	204	6.8	1.3
Clinistix	75	4.2	0.7
Double test (Dreypak and Tes-Tape)	142	8.4	2.0
All tests	654	5.2	1.3

*Subjects already known to have diabetes excluded.

The percentage of positive tests among Tes-Tapes returned was nearly twice that among Dreypaks returned. It is important to note, however, that in terms of percentage of tests *distributed* to the public, the number of positives picked up by Tes-Tape is, if anything, a little

lower than the number detected by Dreypak.

The same effect is seen when the over-all results of the 1956 drive are compared with previous results in Minneapolis (table 3). The 1950 campaign was carried on with liquid samples (without preservative) and Benedict's qualitative test; the other drives used the Dreypak. Though the total number of tests returned in 1956 was low in comparison to most of the previous years, the percentage of positive tests was higher and the total number of positive tests returned exceeded any previous year reported.

TABLE 3

Diabetes Detection Drives in Minneapolis

Year	Total Tests Returned	Positive Tests (Including Known Diabetics) Number	Per cent
1950	24,354	534	2
1951	14,015	258	2
1952	32,063	657	2
1954	9,398	249	3
Twin Cities, 1956	12,565	728	5.8

It seems, therefore, that despite evidence of self-reading of the enzyme tests the number of positive tests reported to the center was not appreciably affected by this. The percentage positive of tests distributed was much the same for Dreypak and Tes-Tape (1.5 per cent and 1.3 per cent, respectively), making it unlikely that any appreciable number of persons, on finding a positive Tes-Tape at home, failed to mail it in. The percentage return of Clinistix tests distributed was lower (0.7 per cent); this may reflect in part the less convenient format of this test, or possibly its relative insensitivity. To be sure, even an unreported positive test will have served a major part of its purpose by calling to the subject's attention the possibility that he or she might have diabetes.

Reliability of the Enzyme Tests in Comparison with the Dreypak: The modified glucose tolerance test described above was used for evaluating the significance of the positive urine tests in persons not previously known to be diabetic. About 60 per cent of those showing glycosuria availed themselves of the test. The results were arbitrarily divided into three groups as follows: normal, 90 mg. per 100 ml. or below; borderline, 91 to 120 mg. per 100 ml.; diabetic, 121 mg. per cent or higher.

Among the double tests in which both Dreypak and Tes-Tape were used on the same specimen, one or both tests were positive for sugar in 142 specimens, but of these only twenty-eight were positive with both tests. Fifty-four tests were positive by Dreypak only and sixty by Tes-Tape only.

Eighty-three persons with one or both urine tests positive reported for blood sugar tests; the urine test results are analyzed according to these results in table 4.

In this relatively small sample, the Tes-Tape appeared to pick up a higher percentage of false positive (normal) results. More significant, probably, is the fact that both the Dreypak and the Tes-Tape missed some diabetic subjects, and in about the same proportion.

However, these differences even out when the entire group of 360 blood sugar results is analyzed in relation to the test used:

With the larger amount of data it is clear there is no difference between the sensitivity of the Dreypak, the Tes-Tape and the Clinistix.

Fading and Late-Developing Enzyme Tests: Since the interval between mailing of the enzyme strip and its reading in the detection center varied anywhere from two days to two weeks, a rough check was made on the significance of tests whose colors faded before they were read and on tests in which the color apparently had developed after being read as negative at home. It was clear in a small number of tests that a test which had faded on the way in still could mean diabetes; also that a test which was negative at home but had developed subsequently usually was normal but also could be indicative of diabetes. However, more complete observations on this point are desirable.

COMMENT

The final validity of the diagnosis depends upon repeated observations of the patient over a period of years, and there is thus some limit to the significance of a short-term study such as the present one. Within this limit, the results indicate that the enzyme tests are neither more nor less sensitive than the Dreypak for large-scale use in diabetes detection. It appears to be unlikely that the enzyme tests will produce an excessive number of false positives, or that any significant number of persons finding a positive test at home will fail to report it to the detection center. The convenience of the enzyme strips is obvious. The format of the Tes-Tape detection unit in particular was well suited to large-scale handling.

The considerable saving in effort and time that the enzyme tests allow could well be applied to the more extensive use of follow-up blood sugar tests such as were used in the present campaign.

TABLE 4

Analysis of double urine test positives according to blood sugar results in eighty-three persons*

	Dreypak Positive (Alone or with Tes-Tape)		Tes-Tape Positive (Alone or with Dreypak)		Both Dreypak and Tes-Tape Positive		All Positives	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Normal	21	42	30	59	7	39	44	53
Borderline	15	30	8	16	1	6	22	27
Diabetic	14	28	13	25	10	55	17	20
Total	50	100	51	100	18	100	83	100

*Subjects already known to have diabetes excluded.

TABLE 5

Analysis of single and double urine test positives according to blood sugar results in 360 persons*

	All Dreypaks (Positive Alone or with Tes-Tape)		All Tes-Tapes (Positive Alone or with Dreypak)		Clinistix		All Positives (Counting Double Tests Once Only)	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Normal	78	47	68	41	24	52	163	45
Borderline	46	27	46	28	11	24	102	28
Diabetic	44	26	50	30	11	24	95	26
Total	168	100	164	100	46	100	360	100

*Subjects already known to have diabetes excluded.

SUMMARY

A comparison was made between a copper-reduction test (Dreypak) and two self-developing enzyme tests for glucose (Tes-Tape and Clinistix) in diabetes detection in Minneapolis and St. Paul. The tests were offered to the public in random fashion through drug stores. In proportion to the number of tests distributed, many more Dreypaks than self-developing tests were returned to the detection center. However, the number of *positive* tests returned in proportion to the number distributed was about the same for Dreypak and Tes-Tape and was somewhat lower for Clinistix. These results suggest (but do not prove) that some self-interpretation of the enzyme tests occurred, though it did not markedly affect the number of tests reported to the detection center.

In the most important part of the study, a modified glucose tolerance test was offered to persons showing a positive urine test; previously known diabetics were excluded. According to the results of the blood sugar tests the subjects were classified as normal, borderline, or diabetic. Of 168 subjects showing positive urine tests with Dreypak, 47 per cent, 27 per cent and 26 per cent were classified as normal, borderline and diabetic respectively. Corresponding results for 164 subjects positive by Tes-Tape were 41 per cent, 28 per cent and 30 per cent, and for forty-six subjects positive with Clinistix 52 per cent, 24 per cent and 24 per cent. These results are essentially identical and indicate that the three tests have about the same sensitivity and reliability when used in diabetes detection.

SUMMARIO IN INTERLINGUA

Le Uso De Simple Tests Enzymatic Pro Glucosa In Le Detection De Diabete: Evalutation Per Medio De Un Modificate Test Del Tolerantia Pro Glucosa

In Minneapolis e St. Paul, un comparation esseva effectuate inter un test a reduction de cupro (Dreypak) e duo auto-disveloppante tests enzymatic (Tes-Tape e Clinistix) pro glucosa in le detection de diabete. Le tests esseva offerite al publico al hasardo via le drogerias. In relation al numero de tests distribuite, le proportion del Dreypaks retornate al centro de detection esseva multo plus grande que le proportion del Tes-Tapes e Clinistixes. Tamen, le numero del tests a reaction positive que esseva retornate in proportion al numero distribuite esseva circa le mesme in le casos de Dreypak e de Tes-Tape e un pauco plus basse in le caso de Clinistix. Iste factos indica possibilemente sed non necessarimente que il occurreva un certe auto-interpretation del tests enzymatic per le individuos, sed isto non afficeva marcatemente le numero de tests reportate al centro de detection.

In le plus importante parte del studio, un modificate test de tolerantia pro glucosa esseva offerite a personas con positive tests del urina. Individuos previemente cognoscite como diabeticos non esseva includite. Super le base del resultatos del tests de sucro sanguinee, le subjectos esseva classificate como normal, casos limite, o diabetic. Ex 168 subjectos con positive tests urinari per Dreypak, le tres classes comprendeva 47, 27, e 26 pro cento, respectivemente. Le cifras correspondente pro 164 subjectos positive per Tes-Tape esseva 41, 28, e 30 pro cento. Pro quaranta-sex subjectos positive per Clinistix, illos esseva 52, 24, e 24 pro cento. Iste resultatos es essentialmente identic. Illos indica que le tres tests es plus o minus equal in sensibilitate e fidelitate quando usate in le detection de diabete.

ACKNOWLEDGMENT

This report is a result of the combined efforts of many physician and lay members of the Twin Cities Diabetes Association of Minneapolis and St. Paul. In addition, special thanks is due to members of the staff of the chemistry laboratory, University of Minnesota Hospitals, who performed the blood sugar determinations, and to Miss Virginia Betlach, who developed and read the Dreypak urine tests. Tes-Tapes were furnished without charge by Eli Lilly and Company and Clinistix tests were donated by Ames Company, Inc.

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Theories of Hunger Sensation

Numerous ideas have been advanced to explain the induction of hunger sensations. One of the early theories, proposed by E. Bulatao and A. J. Carlson (*Am. J. Physiol.* 59:107, 1924) was based on the fact that hunger pangs coincided with the waves of contraction of the empty stomach. They also postulated that hypoglycemia was responsible for this effect. W. W. Scott, C. C. Scott and A. B. Luckhardt (*Am. J. Physiol. 123*:243, 1938) determined blood sugar on human subjects preceding, during, and after normal hunger periods, and found no variations which indicated that the blood sugar level bore a causal relation to the normal hunger periods of man.

J. Mayer (*New Eng. J. Med. 249*:13, 1953) reasoned that it was unlikely that the abundant body stores of protein or fat would decrease much between meals to stimulate the hypothalamic centers. On the other hand, the carbohydrate stores of the body, being small, could easily be depleted. He proposed that hypothalamic "glucoreceptors," sensitive to variations in available blood glucose, were responsible for initiating hunger sensations. Available blood glucose was determined by finding the arteriovenous difference. Since measurement of the arteriovenous blood sugar difference was not possible in the hypothalamic region, the determinations were carried out on peripheral blood with the assumption that the findings were representative of the body generally. Correlation of these findings with hunger periods in subjects on different dietary regimens showed that, on a calorically adequate diet, the arteriovenous (a-v) difference remained high during the day, decreasing only at meal time along with the appearance of hunger feelings. In contrast, hunger appeared earlier on submaintenance diets and the glucose differences became rapidly smaller. However, a recent review on the role of blood sugar and appetite (*Nutrition Reviews 14*:332, 1956) urges caution in the general interpretation of peripherally determined a-v sugar differences.

From *Nutrition Reviews*, Vol. 16, No. 1, pp. 7-8.

Comparison of Benedict's Solution, Clinitest, Tes-Tape and Clinistix

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Benedict's qualitative test for urine sugar has been a standard test for diabetes detection and control since its introduction in 1909. Attempts to obtain a simpler or better test have been numerous. Clinitest was introduced in 1942 and is now used extensively. Recently enzymatic test papers using glucose oxidase[1-3] have been introduced under the names of Clinistix and Tes-Tape. Tes-Tape is represented as being semiquantitative. Clinistix claims only qualitative accuracy, but readings are graded as negative, and faintly, moderately, or strongly positive. Several reports of studies using the enzyme tests have been published. In general, they indicate a fairly good qualitative agreement between copper reduction and glucose oxidase methods. Glucose oxidase test papers were found to be significantly more sensitive than the copper reduction tests, which could conceivably lead at times to insulin overdosage. In addition, glucose oxidase papers have been reported to have little if any value for quantitating the concentration of glucose in the urine although they appear to be reliable and perhaps superior to copper reduction methods for determining the presence or absence of glucose.[4-6]

In this area qualitative urine tests for glucose are very important because for a number of years local practice and teaching have emphasized the use of qualitative urine tests for control of diabetic patients.

This study was undertaken to compare the accuracy of two copper reduction tests (Benedict's and Clinitest) and two enzyme tests (Tes-Tape and Clinistix) in the control of severe juvenile diabetes, employing Sumner's quantitative urine sugar method[7] for reference purposes.

METHODS AND RESULTS

Six hundred and sixty urine specimens obtained from forty-nine juvenile diabetics were tested with Benedict's qualitative test, Clinitest, Tes-Tape, Clinistix, and Sumner's quantitative method. An additional series of 342 urine specimens collected from twenty different juvenile diabetics were tested by Clinitest and Sumner's method by a different observer. Specimens were collected in bottles at four-hour intervals during the day.

The colorimetric method of Sumner[7] was selected as the means of quantitation of sugar in the urines on the basis of more than twenty years of continuous experience during which time we have found it to give consistently reliable results. To test the reliability of this method, glucose in known amounts over the range of concentrations found in diabetic urines was added to a normal urine. Recoveries of the added glucose were within one tenth per cent concentration in every instance. By comparing the results of duplicate analyses, the reproducibility was within one part in fifty, which we consider very satisfactory for the standard colorimetric technic.

The Benedict's qualitative tests were calibrated as follows: negative, 1+, 2+, 3+, and 4+, using a printed color chart* for comparison. A Benedict's test was considered negative unless yellow, orange, or red precipitate was seen distinctly at the bottom of the tube after cooling and settling (about five minutes after the end of a five-minute period in the boiling water bath). When the yellow precipitate at this time was very small in volume (less than 0.1 ml. by visual estimation) the test was classed as 1+. When the yellow precipitate occupied a larger volume, yet upon being remixed with the solution produced a greenish color (rather than yellow, orange or red), the test was classed as 2+. A yellow precipitate at the end of the five-minute heating period, retaining this color when mixed with the solution, was classed as 3+, and a "brick-red" precipitate as 4+. No waiting period to allow for settling is needed when the result is 3+ or 4+.

In the initial series of tests, because of local teaching and practice Clinitest readings were made fifteen seconds after boiling ceased. The admonition to read as 4+

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*Printed color chart for Benedict's qualitative test in Sharp and Dohme Seminar, Vol. 2, No. 4 (1940).

any orange color developing, even during the process of boiling, was ignored. When results were tabulated later, it became apparent that failure to read 4+ on development of an orange color while boiling might have greatly influenced the accuracy of the Clinitest reading. In the second series of urines, readings were made fifteen seconds after boiling ceased, except that any orange color developing during the boiling was read as 4+. Tes-Tape and Clinistix were used according to manufacturer's instructions.

Our comparison may be subject to criticism on the grounds that whereas Sumner's, Benedict's, and Clinitest respond to total reducing substances in the urine, Tes-Tape and Clinistix are specific for glucose. It is possible, by a laborious technic, to eliminate the effect of the non-glucose reducing substances found in urine, but this was not done in the study reported here, desirable though it might be for more accurate evaluation of a specific glucose-oxidase method. At higher concentrations of glucose (above 1,000 mg. per cent) this error may be considered negligible, since it probably amounts to the equivalent of less than 100 mg. per cent of glucose, and the results by Benedict's method should approximate those obtained with enzyme methods, when the Sumner's quantitative method is used for reference purposes.

The data from the initial series are presented in figures 1-4. Each urine specimen was tested by the four qualitative tests and by Sumner's quantitative test. In the figures, for each test indicated, the qualitative test reading, i.e., negative, trace, 1+, etc., is indicated along the baseline and the quantitative result obtained with Sumner's method is indicated on a logarithmic vertical scale, each dot representing a separate test. For instance, a 1+ Benedict's reading that had a reducing substance concentration of 200 mg. per cent by Sumner's method appears in the 1+ column in figure 1 at the level of .20 per cent on the scale. Mean values for each column are indicated.

It was somewhat surprising to find Benedict's test unreliable when glucose concentrations were higher than 500-1,000 mg. per cent (figure 1). Benedict's test appeared useful in the negative, 1+ and 2+ categories, but inadequate in distinguishing the "yellow" 3+ and the "brick-red" 4+ categories; in fact, the mean glucose concentration found by Sumner's method in the urines giving 4+ tests with Benedict's was slightly less than that found in those noted as 3+.

In the initial group (figure 4) when all readings with Clinitest were made after boiling ceased, the 2+, 3+, and 4+ categories did not distinguish different

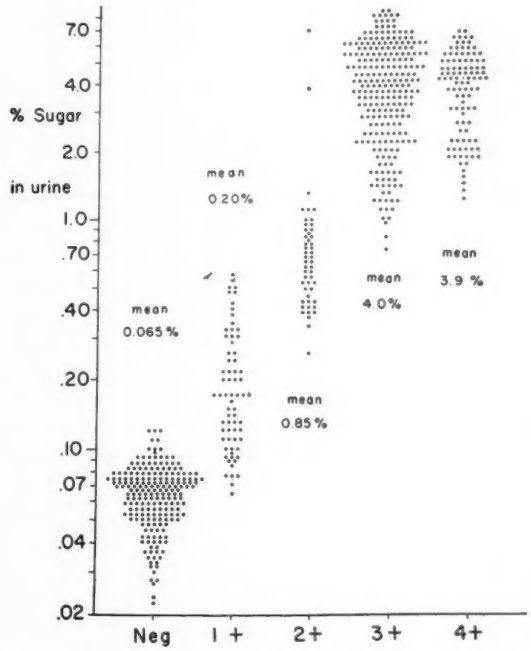

FIG. 1. Benedict's qualitative readings compared with per cent sugar in urine by Sumner method.

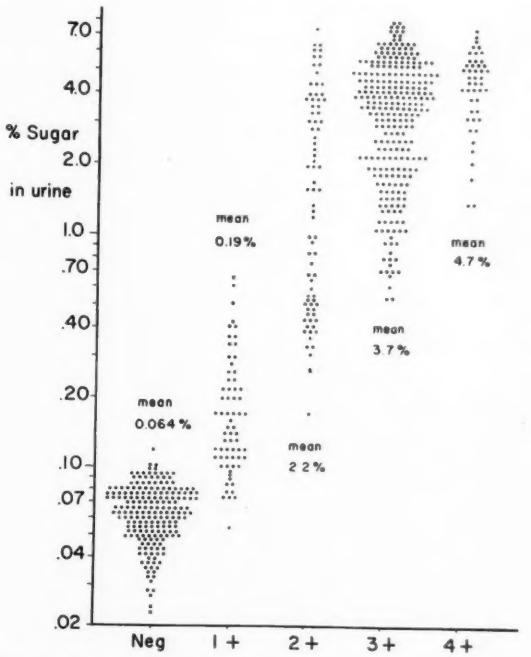

FIG. 2. Tes-Tape readings compared with per cent sugar in urine by Sumner method.

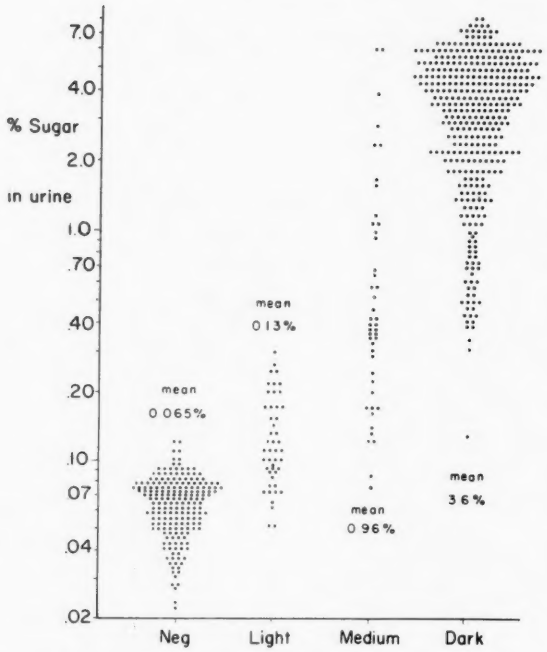

FIG. 3. Clinistix readings compared with per cent sugar in urine by Sumner method.

concentrations of glucose; however, when any orange color developing during or after the process of boiling was read as 4+ (figure 5), a greater differentiation appeared. It is our impression that such misuse of Clinitest is common; in fact, medical students and others in this region have been taught to take the reading after boiling has ceased. Attention should be called to the need for following the fine print instructions carefully, being certain to record any orange color which develops in the process of boiling.

Results with Tes-Tape are given in figure 2. The negative and 1+ tests were consistently related to the quantitative glucose findings. On the other hand, the wide range of glucose concentrations in urines showing colors read as 2+, 3+, and 4+ indicated a lack of useful purpose in designating such categories. There seems to be no indication, therefore, for denoting color observations above the 2+ level with this test.

When testing Clinistix (figure 3), the negative, "light," and "dark" results appeared to have practical value, when comparison was made with Sumner's method. The pattern of "medium" tests, however, suggests that this classification could well be omitted and included in either the "light" or "dark" classification instead. It is to be noted that Clinistix avoids the criticism concerning quantitative efficacy directed at the

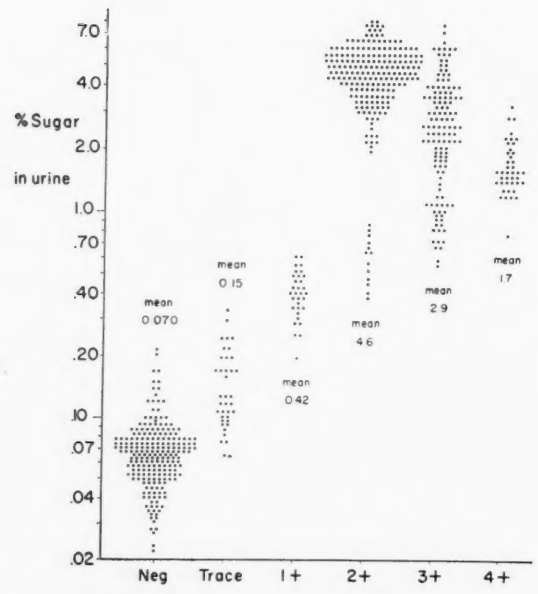

FIG. 4. Clinitest readings (fifteen seconds after boiling is completed) compared with per cent sugar in urine by Sumner method.

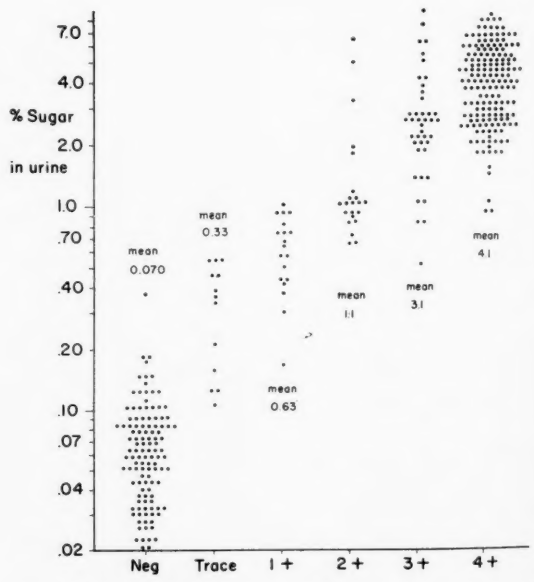

FIG. 5. Clinitest readings (scoring transient orange color, while boiling, as 4+) compared with per cent sugar in urine by Sumner method.

other three methods because the manufacturer makes no claim that this test is able to distinguish varying concentrations of glucose above a few hundred milligrams per cent.

SUMMARY

On the basis of our observations, it appears that all four of the tests for urine glucose employed in this study have a satisfactory accuracy on urine specimens which they designate as negative. At lower levels of positivity (1+ or 2+ Benedict's, trace or 1+ Clinitest, 1+ Tes-Tape, "light" Clinistix) all four tests are consistently related to the quantitative findings by Sumner's method. At higher glucose concentrations, as determined by Sumner's method, Benedict's, Clinitest (read fifteen seconds after boiling ceased), Tes-Tape and Clinistix became unreliable. It would, therefore, appear that these tests for urine glucose might well be re-evaluated, with consideration given to our observation that with the exception of a 4+ Clinitest read as any orange color developing during or after boiling, no useful purpose is served by attempts to obtain quantitative reports on urine specimens containing more than 500 to 1,000 mg. per cent glucose: thus, Benedict's might be calibrated as negative, 1+, 2+, and 3+; Clinitest as negative, trace, 1+, 2+, 4+; Tes-Tape as negative, 1+, 2+; and Clinistix as negative, "light," "dark."

This study was deliberately performed on labile juvenile diabetic patients where it is often important to have fairly accurate urine glucose determinations throughout the day in order to avoid insulin reactions or poor control. The Benedict's qualitative test and Clinitest appeared somewhat more reliable in distinguishing glucose concentrations near 500 mg. per cent from those higher than 1,000 mg. per cent, and the Clinitest was the only test wherein a certain color change clearly indicated a glucose concentration higher than 2,000 mg. per cent. Therefore, in "brittle" patients who easily slip from insulin reaction to acidosis, especially when the "sliding scale" method of determining insulin dosage is being used, it might be wisest to continue the use of these two methods at this time. In the more stable diabetic any of the four tests described would be adequate.

It would appear undesirable to change back and forth from one urine testing method to another for two reasons. The significance of a 3+ Benedict's or Clinitest is entirely different from the significance of a 3+ Tes-Tape; in the former the glucose concentration would average over 1,000 mg. per cent, while in the latter, according to the manufacturer, the glucose concentration would average under 1,000 mg. per cent. In addition, colors are reversed by the tapes; instead of a dark blue color indicating negative or very low glucose concentrations, as with Benedict's or Clinitest, a dark blue Tes-Tape indicates very high concentrations of glucose. These factors might easily cause confusion and disaster in a severe diabetic patient.

The relative value of the above tests for detection of diabetes is a different problem. This has been studied and is being reported separately.[6]

SUMMARIO IN INTERLINGUA

Comparation De Solution De Benedict, Clinitest, Tes-Tape, E Clinistix

Super le base de nostre observationes, il pare que omne le quatro tests del glucosa urinari, le quales esseva usate in iste studio, attinge grados satisfactori de accuratia in le caso de specimens de urina que illos characterisa como negative. A basse nivellos de positivitate—1+ o 2+ (Benedict), tracia o 1+ (Clinitest), 1+ (Tes-Tape), e clar (Clinistix)—omne le quatro tests es regularmente correlationate con le valores quantitative trovate secundo le methodo de Sumner. Ubi le methodo de Sumner revelava plus alte concentrationes de glucosa, solution de Benedict, Clinitest (legite 15 secundas post que le ebullition cessava), Tes-Tape, e Clinistix perdeva lor fidelitate. Per consequente, il pare desirabile que iste tests pro glucosa urinari es re-evalutate, prendente in consideration nostre experientia que—con le exception del possibilitate de leger como 4+ omne coloration orange que se disveloppa in Clinitest durante o post le ebullition—nulle objectivo practic es servite per le essayo de obtener, per medio de iste tests, resultatos quantitative in le caso de specimens de urina que contine plus que 500 a 1.000 mg de glucosa per 100 ml. Assi il es forsan indicate calibrar le test de Benedict como "negative, 1+, 2+, e 3+," Clinitest como "negative, tracia, 1+, 2+, e 4+," Tes-Tape como "negative, 1+, e 2+," e Clinistix como "negative, clar, e obscur."

Iste studio esseva executate deliberatemente con labile diabeticos de juvene etates in qui il es frequentemente importante obtener accurate determinationes del glucosa urinari in le curso del die pro evitar reactiones a insulina o le consequentias de dysregulation. Le test qualitative de Benedict e Clinitest esseva apparentemente plus efficace in distinguer concentrationes de glucosa proxime a 500 mg per 100 ml ab concentrationes de plus que 1.000 mg per 100 ml, e Clinitest esseva le sol del quatro tests studiate in que un certe alteration del color indicava clarmente un concentration de glucosa de plus que 2.000 mg per 100 ml. Assi, in patientes "delicate," qui

transi facilemente ab le reaction insulinic al stato de acidosis, il es probabilemente a recommendar que le uso del duo mentionate methodos es continuate pro le tempore presente, specialmente si le dosages de insulina es determinate per medio del methodo a "scala mobile." In le caso del plus stabile diabeticos, non importa le qual del quatro tests describite es adequate.

Il es probabilemente pauco desirabile alternar inter plure methodos pro testar le urina. Pro isto il existe duo rationes. Le signification de 3+ in le test de Benedict o in Clinitest es completemente differente ab le signification de 3+ in Tes-Tape. In le prime de iste duo casos, le concentration de glucosa haberea un nivello medie de plus que 1.000 mg per 100 ml, durante que in le caso de Tes-Tape—secundo le assertiones del fabricante—le concentration de glucosa haberea un nivello medie de minus que 1.000 mg per 100 ml. In plus, le signification del colores es invertite inter le varie bandas. In loco del negativitate o multo basse concentration de glucosa que es indicate per un color blau obscur in le caso de Benedict o Clinitest, un color blau obscur in Tes-Tape indica multo alte concentrationes de glucosa. In patientes con diabete de alte grados de severitate, iste factores poterea devenir le causa de confusion e mesmo de disastros.

Le valor relative del hic-discutite tests pro deteger diabete es un altere problema. Illo ha etiam essite studiate, e un reporto separate ha essite preparate pro illo.

ACKNOWLEDGMENT

This study was aided by grants from Ames Company, Inc., and from Eli Lilly and Company. Clinitest and Clinistix were supplied by Ames Company, Inc. Tes-Tape was supplied by Eli Lilly and Company.

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I must allow myself a further digression to recall that this contact with the insulin problem marked also the beginning of another of those enduring friendships, which have meant so much to me both scientifically and personally. Even on my first visit to Toronto, an understanding seemed to grow naturally with the junior partner in the historic insulin enterprise, Charles H. Best, that he would come over to work with me as soon as he had completed his medical qualification. So I was to find myself, in due coursse, working with Best on the detailed mechanism of the action of insulin—my only adventure, I think, into problems of metabolism—and, at a later visit, on what was, for me, the less novel ground of the natural occurrence of histamine, in the different organs of the body.

By Sir Henry Dale in *Perspectives in Biology and Medicine,* Vol. 1, No. 2, Winter 1958, University of Chicago Press.

Criticism in Medicine and the Biological Sciences

Some of us remember the awe and respect that surrounded Dr. Anton Carlson because of his pungent criticism. It was devastating and accurate. It had the salutary effect of making the old speaker check his references and the young speaker stay on his toes and not say foolish things or make sweeping generalizations. One often hears the expression today that constructive criticism is valuable while destructive criticism is harmful. Criticism is, of its nature, destructive. The notion of constructive criticism probably arose because criticism may be delivered in an effective way which cuts with small pain. It can have a result which is constructive if it leads to the correction of error, the improvement of technique, and the *debridement* of those necrotic evils of obscure style, sterile speculation, and discord between evidence and conclusion which so beset us.

By William B. Bean, from *Perspectives in Biology and Medicine,* Vol. 1, No. 2, Winter 1958, University of Chicago Press.

Special Article

Recent Statistics on Diabetes

The death rate from diabetes in 1957 was higher than in both 1956 and 1955. For the United States as a whole provisional figures for the year 1957, based upon a 10 per cent sample of death certificates, showed a 4 per cent increase over 1956 (table 1). In the urban population of the United States, represented by Industrial policyholders of the Metropolitan Life Insurance Company, the death rate was 2 per cent above that for 1956. The rise in mortality attributed to diabetes was concentrated in the last quarter of the year when the death rate from the disease was over 15 per cent higher than in the corresponding period of 1956. For the first nine months, the rates for the two years were approximately the same.

The high mortality in the last quarter of 1957, which, moreover, has extended into the first quarter of 1958, reflected the prevalence of influenza, especially the so-called Asiatic strain. The widespread outbreak in October and November brought a rise in the general death rate as well as that from pneumonia and influenza. Furthermore, the high mortality in the United States resulting from the influenza outbreak was largely concentrated at the older ages, the principal victims being those suffering from chronic diseases of later life, among them diabetes.

In the states and cities of the United States regularly contributing data to the Committee on Statistics, increases in diabetes mortality for 1957 were recorded for New York City and Baltimore; Philadelphia showed no change, but the rates for New York State, Maryland and Boston were below the preceding year.

Diabetes mortality in the two large Canadian cities, Toronto and Montreal, showed divergent trends. The rate for Montreal rose 13 per cent, but that for Toronto fell 14 per cent. It may be noted that the impact of the influenza epidemic was less in Canada than in this country.

In England and Wales the death rate from diabetes in the first nine months of 1957 was appreciably lower than for the corresponding period of 1956. In the last quarter of 1957, however, when England was experiencing an influenza epidemic, deaths from diabetes exceeded the number in the last quarter of 1956, the increase being especially large among females. For the year as a whole, the death rate from diabetes in 1957 was 4 per cent less

Submitted by the Committee on Statistics, Herbert H. Marks, Chairman. The Committee welcomes suggestions or actual materials suitable for this section in future issues from Association members and other readers of the Journal.

TABLE 1

Recent data on diabetes mortality
Deaths and death rates—1957 and 1956

Area	Death Rates Per 100,000		Number of Deaths	
	1957	1956	1957	1956
United States (10 per cent sample)	16.4	15.8	2,793	2,634
Metropolitan Life Insurance Company Industrial Policyholders	15.2	14.9	2,657	2,649
New York State	18.2	19.2	2,985	3,109
New York City	19.4	18.9	1,582	1,527
Maryland	17.3	18.4	500	511
Baltimore	26.0	25.1	255	244
Boston	14.3	18.1	118	148
Philadelphia	20.7	20.7	450	450
Toronto	17.3	20.2	114	130
Montreal, Resident	17.9	15.8	202	175
London (Administrative County)	7.5	8.2	245	267
England and Wales				
Total	7.0	7.3	3,139	3,242
Males	4.7	5.1	1,014	1,108
Females	9.1	9.2	2,125	2,134

Note: Rates for the states and cities are based upon local estimates of population. United States data are based upon returns from a 10 per cent sample of death certificates received in vital statistics offices, as published in "Current Mortality Analysis," a monthly report of the National Office of Vital Statistics of the U. S. Public Health Service.

than in 1956, due largely to the decline in the rate for males. In London (Administrative County) the rate also fell 9 per cent in 1957 as compared with 1956. Even for the last quarter of the year the rate in 1957 was less than in 1956, although the influenza outbreak brought a rise in the total death rate in London. Apparently many deaths of diabetics during the epidemic were ascribed to influenza. It may be noted that deaths ascribed to diabetes accounted for only 0.5 per cent of the city's deaths in the last quarter of 1957.

Regional mortality for diabetes in the United States for 1957 based upon the 10 per cent sample of death certificates likewise showed no consistent pattern (table 2). Five of the nine areas showed increased rates in 1957 as compared with 1956 ranging from 7 per cent to 28 per cent, while four areas showed declines ranging from 2 per cent to 12 per cent. The largest increase was recorded in the West South Central area; the largest decrease for the Rocky Mountain area, but the number of deaths in the sample there was small. It is possible that the complete figures will show somewhat greater uniformity geographically in the comparison of 1956 and 1957 rates.

Complete and final mortality data from diabetes by states are now available for the year 1955 and are shown together with the rates for the two preceding years in table 3. Death rates are still highest in the Northeastern section of the country and lowest in the South and in the Far West, with the Middle Western states generally showing rates of intermediate size. In 1955 the highest death rate was recorded in New Hampshire, with only a slight lead over Rhode Island which for many years had ranked first. New Mexico had the lowest death rate from the disease in 1955 and Arizona next lowest. These two states likewise have for many years experienced the minimum rates recorded in the country. Only slightly higher were the rates in Tennessee and Alabama. The maximum figure for New Hampshire is somewhat over three times that of the minimum rate of New Mexico. On a regional basis the highest rate was recorded in the Middle Atlantic states, with New England next in line; the lowest in the East South Central states and next lowest in the Pacific Coast states. Within regions there are, in many cases, rather wide differences. In the Mountain states, Montana's rate was more than double that for New Mexico; in the South Atlantic states, Delaware's rate was double that for North Carolina; and in New England, the East North Central and the West North Central areas, the maximum rate in the area was about one and one half times the minimum.

The level of the rates for the individual states is affected by a number of factors. Most easily measurable of these is the composition of the population by age, the effect of which can be measured by the use of age-adjusted rates. For that purpose, however, it is necessary to use mortality figures for the period of years close to the last census. Such data, the first of the kind on the basis of the Sixth Revision of the International List, have recently been released by the National Office of Vital Statistics. Table 4 gives age-adjusted death rates by color and sex for each state in 1949-1951. For comparison, the crude death rates for the total population are given in the table. It will be seen first that the range of the adjusted death rates was appreciably less than that

TABLE 2

Number of deaths and death rates from diabetes in geographic division: United States reporting area for the 10 per cent sample: 1957, 1956 and 1955

Geographic Division	Death Rates per 100,000*			Number of Deaths*		
	1957	1956	1955	1957	1956	1955
U. S. reporting area	16.4	15.8	15.3	2,793	2,634	2,506
New England	20.9	19.5	19.1	206	190	184
Middle Atlantic	20.9	22.2	20.9	681	717	674
East North Central	19.0	19.4	18.1	666	668	609
West North Central	17.1	15.1	13.3	262	228	197
South Atlantic	14.1	11.3	12.6	348	271	295
East South Central	11.5	11.8	9.2	136	138	107
West South Central	13.4	10.5	12.1	218	168	190
Mountain	10.8	12.3	10.6	69	76	63
Pacific	11.2	10.0	10.6	207	178	187

*Excludes Armed Forces overseas.

Note: These data from the 10 per cent sample are subject to sampling error. The number of deaths, as given, does not cover the entire United States for each month but is limited by the completeness of the reporting area. The size of the reporting area is indicated by the footnote on page 7 of each monthly issue of the "Current Mortality Analysis."

Source: Data furnished by National Office of Vital Statistics of the U. S. Public Health Service.

TABLE 3

Death rates per 100,000 from diabetes in the United States by geographic region and state,* 1955, 1954 and 1953

Region and State	1955	1954	1953
United States	15.5	15.6	16.3
New England	20.0	19.4	19.7
Maine	17.7	17.9	15.1
New Hampshire	26.6	21.8	21.1
Vermont	15.1	15.6	19.4
Massachusetts	20.5	19.6	19.0
Rhode Island	26.2	24.6	30.0
Connecticut	17.0	17.7	19.0
Middle Atlantic	21.1	20.8	22.5
New York	20.2	19.9	21.3
New Jersey	21.0	21.0	21.2
Pennsylvania	22.5	22.0	24.7
East North Central	17.9	19.0	19.5
Ohio	21.4	22.8	23.3
Indiana	16.7	16.9	17.6
Illinois	13.9	15.4	16.7
Michigan	19.4	20.5	20.5
Wisconsin	17.6	18.7	18.4
West North Central	15.8	16.3	17.3
Minnesota	16.2	17.8	17.4
Iowa	16.8	15.3	16.7
Missouri	15.2	15.3	16.8
North Dakota	12.8	13.9	15.5
South Dakota	13.3	15.5	17.8
Nebraska	19.2	18.6	21.2
Kansas	14.4	16.4	16.6
South Atlantic	12.2	11.9	12.3
Delaware	20.0	18.6	22.1
Maryland	17.7	16.9	17.6
District of Columbia	12.0	14.0	13.7
Virginia	11.3	10.3	10.1
South Atlantic (continued)			
West Virginia	13.2	12.4	11.5
North Carolina	9.7	10.0	10.4
South Carolina	10.7	10.5	12.0
Georgia	11.2	10.6	11.9
Florida	12.7	12.8	12.4
East South Central	10.2	10.5	11.0
Kentucky	12.4	13.0	12.8
Tennessee	9.2	8.7	9.2
Alabama	9.2	9.7	10.8
Mississippi	10.1	10.9	11.6
West South Central	11.5	11.5	11.6
Arkansas	10.7	10.1	9.7
Louisiana	14.5	14.3	14.8
Oklahoma	13.8	13.5	14.2
Texas	10.1	10.2	10.3
Mountain	11.3	9.9	10.7
Montana	17.6	15.8	14.7
Idaho	12.7	11.5	13.1
Wyoming	14.1	9.1	14.1
Colorado	10.2	9.8	11.0
New Mexico	7.9	7.2	5.4
Arizona	8.9	7.1	8.2
Utah	12.7	11.7	12.0
Nevada	10.2	6.7	11.2
Pacific	10.9	10.4	10.9
Washington	13.9	14.3	15.3
Oregon	10.1	10.3	12.4
California	10.4	9.7	9.9

*By place of residence. Excludes Armed Forces overseas.
Source: National Office of Vital Statistics of the U. S. Public Health Service. Special Reports—National Summaries.

for the crude rates. The maximum rate for Rhode Island (28.1) is approximately three and a half times that of the minimum recorded in California (7.9). As against this the maximum crude rate of Rhode Island was more than five times the minimum of 6.7 for New Mexico.

The effect of age adjustment of the rates is further seen by the relative levels of these rates both in comparisons between regions and within regions. Thus, the crude rates in several Northern and Eastern states were generally higher than those in the Middle West, but the age-adjusted rates were more nearly equal, and in some cases the relative positions were reversed. For example, the crude rate for New York State in 1949-1951 was 6 per cent above that for North Dakota, but its age-adjusted rate was 2 per cent less. Again, New York's crude rate was 55 per cent above that for South Carolina, although the age-adjusted rates were identical for the two states. Again South Carolina's crude rate was about identical with that for Florida, but its age-adjusted rate was 40 per cent higher than Florida's. While the age-adjusted rate for California was the lowest in the country in 1949-1951, four other states had crude rates as low or lower than California's. New Mexico's crude rate was 27 per cent less than California's, although the age-adjusted rate was 10 per cent higher.

The table brings out many striking differences of distribution of diabetes mortality for the country as a whole and for the several areas of the country. Among the more notable items are these: The increase in the proportion of older persons in the population results in a very sizable difference, 12 per cent, between the crude rate (16.5) and the rate age-adjusted (14.5) on the basis of the 1940 population.

In the population as a whole the female age-adjusted rate was nearly one and a half times that of males. Among whites the excess of the female rate was about 50 per cent, but among nonwhites the female rate was nearly double that for males. The nonwhite rate was about 20 per cent higher than the white rate, but this difference was virtually all accounted for by the excess of more than

TABLE 4

Crude and age-adjusted death rates per 100,000 from diabetes mellitus for total persons and age-adjusted rates by color and sex, United States and each state, 1949-51

Area	Crude Rates Total persons	Age-Adjusted Death Rates Total			White			Nonwhite		
		Both sexes	Male	Female	Both sexes	Male	Female	Both sexes	Male	Female
United States	16.5	14.5	11.5	17.4	14.3	11.4	16.9	17.0	11.5	22.4
New England										
Maine	18.6	13.9	11.4	16.2	13.9	11.4	16.2			
New Hampshire	21.8	15.1	14.1	16.2	15.2	14.1	16.3			
Vermont	18.3	13.7	11.7	15.7	13.7	11.7	15.7			
Massachusetts	21.6	16.0	13.4	18.1	15.9	13.3	18.0	22.6		25.6
Rhode Island	35.1	28.1	20.2	34.7	28.0	20.1	34.5			
Connecticut	19.2	15.5	11.0	19.6	15.4	10.9	19.4			
Middle Atlantic										
New York	19.5	15.9	12.3	19.1	15.6	12.2	18.7	20.2	13.1	26.2
New Jersey	20.8	17.6	12.4	22.2	17.2	12.3	21.4	24.8	13.1	36.5
Pennsylvania	21.9	18.5	12.3	24.3	18.3	12.2	24.0	20.9	14.1	27.9
East North Central										
Ohio	23.0	18.9	14.4	23.2	18.5	14.3	22.5	24.5	14.5	34.9
Indiana	17.8	14.5	11.8	17.2	14.4	11.8	16.8	17.6		24.0
Illinois	21.4	17.4	13.3	21.4	17.4	13.4	21.1	16.4	9.4	23.5
Michigan	22.9	21.7	16.5	27.0	21.3	16.4	26.3	26.2	15.5	37.8
Wisconsin	18.9	15.2	12.1	18.3	15.2	12.2	18.1			
West North Central										
Minnesota	16.8	13.3	11.2	15.6	13.3	11.2	15.5			
Iowa	17.0	12.1	10.3	13.9	12.1	10.3	13.8			
Missouri	17.9	13.2	10.5	15.6	12.7	10.3	14.9	19.1	13.4	24.7
North Dakota	18.4	16.3	13.1	20.1	16.3	13.2	20.0			
South Dakota	17.7	14.7	13.8	15.7	14.4	13.7	15.3			
Nebraska	18.7	13.7	11.9	15.5	13.7	11.9	15.5			
Kansas	17.5	12.8	11.3	14.2	12.7	11.4	13.9	15.4		
South Atlantic										
Delaware	24.7	21.6	13.2	29.3	20.0	12.8	26.3	33.1		53.1
Maryland	17.7	17.8	12.9	21.9	17.6	13.2	21.1	18.1	10.6	26.1
District of Columbia	14.0	13.3	12.9	13.6	11.6	12.6	10.8	17.3	13.0	21.0
Virginia	11.0	12.0	9.2	14.5	10.5	9.0	11.7	17.4	10.0	25.0
West Virginia	12.8	13.0	8.8	17.5	12.7	8.8	16.8	17.0		28.1
North Carolina	9.8	12.0	11.0	12.9	11.0	10.7	11.2	15.4	11.8	18.8
South Carolina	12.6	15.9	14.4	17.4	15.1	14.9	15.4	18.2	14.3	21.4
Georgia	11.8	13.2	11.8	14.5	11.5	11.8	11.4	17.7	12.2	22.6
Florida	12.7	11.3	9.3	13.1	9.8	8.7	10.8	16.8	11.1	22.6
East South Central										
Kentucky	12.3	11.3	9.7	12.8	10.5	9.3	11.7	20.2	14.1	26.1
Tennessee	9.2	9.3	8.2	10.5	8.2	7.8	8.6	14.9	9.9	19.8
Alabama	10.2	11.3	10.4	12.2	9.9	10.0	9.7	15.0	11.6	18.0
Mississippi	11.4	12.0	9.7	14.4	10.3	9.0	11.5	14.5	10.7	18.3
West South Central										
Arkansas	9.9	9.4	7.8	11.0	8.6	7.3	9.9	12.2	9.6	14.9
Louisiana	13.4	14.4	10.1	18.4	13.3	9.5	16.7	17.5	12.0	22.7
Oklahoma	13.5	11.4	10.1	12.6	10.8	9.9	11.8	17.6	13.0	22.2
Texas	11.0	11.6	9.9	13.2	11.2	10.0	12.4	13.8	9.4	18.2
Mountain										
Montana	14.3	12.0	8.3	16.7	12.0	8.4	16.6			
Idaho	13.6	13.0	12.3	13.8	12.9	12.3	13.7			
Wyoming	12.9	13.7	8.8	19.7	13.6	8.8	19.5			
Colorado	11.5	9.6	8.9	10.3	9.6	9.0	10.2			
New Mexico	6.7	8.7	6.9	10.7	8.6	7.2	10.1			
Arizona	8.3	9.5	7.4	11.8	9.3	6.9	11.8			
Utah	11.3	12.6	10.3	15.0	12.7	10.6	14.8			
Nevada	12.9	12.7	11.4	14.7	12.9	12.0	14.1			
Pacific										
Washington	14.8	12.2	10.6	14.0	12.1	10.5	13.8			
Oregon	11.8	9.7	7.9	11.6	9.6	8.0	11.4			
California	9.2	7.9	7.4	8.5	7.8	7.3	8.3	10.0	9.4	10.7

Note: Age-adjusted on the basis of the age distribution of the population of the United States as enumerated in 1940. For nonwhites the death rates are not shown in states with less than thirty deaths in 1949-51.

Source: National Office of Vital Statistics.

30 per cent among nonwhite females compared with white females. The rates for white and nonwhite males were approximately the same.

The excess of the death rate of nonwhite females as compared with white females was found in virtually every state where there were sizable nonwhite populations. In several Northern states extremely high rates were found among the nonwhite female population—53.1 in Delaware, 37.8 in Michigan, 36.5 in New Jersey and 34.9 in Ohio. In all these states these rates were 40 per cent or more above the corresponding rates for white females. Even in the South the margin between the diabetes mortality of females of the two races was wide, with rates for the nonwhite females double or more that for white females in several cases.

The excess of female over male rates was quite general; among whites, the only exceptions were Alabama, Georgia and the District of Columbia, where the male rate was slightly the higher. The range of difference among whites was fairly marked—from less than 20 per cent in several states, chiefly in the South, to 75 per cent or more in some others—Connecticut, Pennsylvania, Delaware, West Virginia, Montana and Wyoming.

The latest detailed statistics on mortality from diabetes in the United States by color, sex and age, for the year 1955, are given in table 5. In the aggregate and for all color-sex groups except nonwhite females, the death rates rose steadily through adult life to a maximum at ages eighty to eighty-four. Among nonwhite females the peak was reached at ages sixty-five to sixty-nine and fell appreciably in the later age groups. Among white persons the death rates at the younger childhood ages were the same for both sexes, but in the teens the female rates were somewhat the higher. From ages twenty to forty-nine the male rates were the higher, the difference being especially marked at ages thirty-five to forty-four. At ages fifty and over the female rates were distinctly the higher. Among nonwhites the number of deaths in the age groups under thirty-five was too few to reveal any sex difference in mortality. From ages thirty-five on, the female death rates were greatly in excess of those of males—more than double between ages forty-five and sixty-nine. Comparison of the mortality of whites with nonwhites showed that among males the nonwhites were the higher from ages twenty-five through sixty-nine and among females from ages twenty-five through seventy-four. The differences were particularly large among females between ages thirty-five and fifty-nine where the rates were several times as high among the nonwhite as among the white. The figures would indicate that the

TABLE 5

Number of deaths and death rates per 100,000 from diabetes mellitus by race, sex and age. United States, 1955. (Excludes Armed Forces overseas)

Age Period (Years)	Death Rates					Deaths				
		White		Nonwhite			White		Nonwhite	
	Total	Male	Female	Male	Female	Total	Male	Female	Male	Female
All Ages	15.5	12.8	18.5	9.7	18.6	25,488	9,242	13,714	840	1,692
Under 1	0.6	0.6	0.3	2.0	1.6	23	10	4	5	4
1-4	0.3	0.3	0.3	0.3	0.1	42	21	17	3	1
5-9	0.3	0.3	0.3	0.1	0.2	51	24	24	1	2
10-14	0.5	0.4	0.5	1.0	0.9	64	23	26	8	7
15-19	0.7	0.4	0.8	1.3	1.1	75	19	39	9	8
20-24	1.4	1.4	1.3	1.4	2.0	141	59	61	8	13
25-29	2.1	2.3	1.9	2.8	2.6	248	115	98	17	18
30-34	2.6	2.7	2.3	3.9	3.2	320	143	131	24	22
35-39	3.2	3.6	1.9	7.3	7.9	374	185	102	39	48
40-44	4.5	4.5	3.0	8.1	13.2	503	224	155	44	80
45-49	7.7	7.1	5.9	8.0	30.3	777	318	270	38	151
50-54	14.4	12.1	12.4	20.9	48.6	1,270	476	501	86	207
55-59	27.0	21.9	27.6	31.8	70.9	2,123	769	1,013	105	236
60-64	49.6	37.9	56.7	45.9	102.4	3,318	1,143	1,804	113	258
65-69	82.9	60.6	100.3	65.0	146.4	4,433	1,455	2,593	117	268
70-74	111.6	91.8	129.2	76.4	137.0	4,538	1,615	2,641	97	185
75-79	145.0	127.7	165.2	84.3	110.1	2,689	1,363	2,158	70	98
80-84	168.8	154.2	187.6	97.6	115.9	2,238	834	1,313	40	51
85 +	160.0	150.3	183.4	43.8	81.0	1,251	442	761	14	34
Not stated						10	4	3	2	1

Source: United States Department of Health, Education and Welfare, Public Health Service, National Office of Vital Statistics Special Reports—National Summaries, volume 46, number 5, May 6, 1957.

duration of life of nonwhite diabetics is less than that of whites and perhaps, too, that the frequency of undetected diabetes is fairly high among elderly nonwhites, especially the females.

The World Health Organization regularly compiles available vital statistics from countries all over the world and publishes them in some detail in its annual *Epidemiological and Vital Statistics*. Table 6 has been assembled from the annual volumes containing the statistics for 1952 to 1954 for those countries which furnish the statistics on diabetes mortality by sex. The international differences in the mortality recorded in the table cannot be taken at face value because the level of the diabetes rates in the different countries is influenced by many factors such as the age composition of the population, differences in point of view of physicians with regard to the designation of the cause of death of diabetics, and the quality and abundance of the facilities for diagnosis and treatment.*

In 1954 the range of mortality displayed in the table was rather large—from 2.2 to 12.4 per 100,000 among males and from 2.4 to 18.7 for females. For Western Europe alone the range was from 3.3 to 9.5 among males and from 6.0 to 17.4 among females.

The United States had the highest recorded death rate both among males and females. The rate in this country was about one and one half times that for Canada. Of the European countries, Switzerland experienced the highest rate in both sexes, while that for Finland was lowest among the males and that for Portugal lowest among the females. There were surprisingly large differences between countries with populations of the same ethnic group. For example, the rates for Sweden were distinctly higher than those for Norway and Denmark. The rates for England and Wales were comparatively low while those for France and Italy relatively high. The rates among males in Italy averaged slightly higher than those for French males, but the reverse was true among females. Rather surprising was the low rate for the Jewish population of Israel. The classification of the causes of death among diabetics may be in part responsible, but so are such factors as the low average age of the population, the large increase in the population drawn from non-European areas, and the high proportion of the population engaged in agricultural and manual work. The economic and occupational distribution of Jews in Israel is quite different from that among those in western countries.

*For a more detailed statement on this matter, see World Health Organization—Epidemiological and Vital Statistics Report, vol. 8, no. 11, 1955, p. 467.

TABLE 6

Death rates per 100,000 from diabetes in various countries, by sex, 1952-1954

	Males			Females		
Country	1954	1953	1952	1954	1953	1952
United States	12.4	12.7	13.0	18.7	19.8	19.7
Canada[1]	8.4	8.8	8.9	12.9	13.2	13.1
Austria	4.8	5.3	5.9*	9.3	8.3	8.9*
Denmark	4.7	4.4	3.9	7.2	5.7	6.7
Finland	3.3	4.9	3.9	7.8	7.4	7.9
France	8.0	8.3	7.7	14.4	14.6	13.6
Germany Federal Republic	7.3	7.7	8.3	12.5	13.3	13.7
Italy	8.5	8.2	7.9	12.6	11.9	11.6
Norway	5.5	5.1	6.1	8.1	7.4	7.6
Netherlands	7.4	6.5	7.1	16.3	16.0	15.7
Portugal	5.5	4.3	4.6	6.0	5.5	6.0
England and Wales	4.9	5.0	5.2	8.6	9.3	9.8
Scotland	5.2	5.3	5.8	11.8	11.6	12.5
Northern Ireland	5.3	4.4	4.3	9.6	7.2	6.5
Sweden	7.7	7.8	9.6	12.8	14.0	14.3
Switzerland	9.5	11.1	10.1	17.4	18.4	17.6
Israel[2]	3.0	2.8	3.2	3.5	3.3	4.1
Australia[3]	8.2	8.1	8.7	16.3	17.0	16.5
New Zealand[4]	7.3	8.7	9.7	12.4	16.5	13.9
Ceylon	8.7	8.4	8.9	4.9	4.8	4.4
Japan	2.2	2.4	2.2	2.4	2.5	2.4

[1]Excludes Yukon and Northwest Territories.
[2]Jewish population.
[3]Excludes full-blood aboriginals.
[4]Excludes Maoris.
*5th Revision (1938).
Source: World Health Organization—Annual Epidemiological and Vital Statistics, 1952, 1953 and 1954.

Among males in Australia and New Zealand the death rate from diabetes was of the same order as in Canada, but among females somewhat higher. In these three Commonwealth countries, the rates were well above those recorded in Great Britain.

Japan recorded the lowest death rate of diabetes of all the countries listed. In Ceylon the reported rates for males were relatively high, in sharp contrast with the situation among females.

Except for Ceylon, the rates among females were uniformly higher than among males. The sex ratio of the death rate varies widely; it is comparatively low in Japan and Israel, whereas in Finland, the Netherlands, Scotland and Australia the female rate was about double the male rate.

No consistent trend was present in the mortality from diabetes over the three-year period in the various countries included in the table. It may be noted, however, that the 1954 rates were lower than those for 1952 in the majority of countries both among males and females.

EDITORIALS

RECENT REPORTS ON THE MECHANISM OF THE ACTION OF THE ARYLSULFONYLUREAS

The current status of this problem was discussed in an editorial in the January-February 1958 issue of DIABETES.[1] Three recently published papers presenting new data warrant further comment.

The β-cytotropic hypothesis of the mechanism of action of these hypoglycemic drugs is gaining increased validity. It postulates that they stimulate the β cells of the pancreas to secrete an extra amount of insulin into the portal vein which exerts its first action upon the liver. The liver, in consequence, diminishes its output of glucose into the blood by: (1) increasing glycogen storage; (2) decreasing formation of glucose from protein or other precursors; or (3) stimulating lipogenesis from carbohydrate. Hypoglycemia results. Unless the secreted insulin is completely bound by the liver so that none of it is free to reach the systemic circulation, it would be expected that the utilization of carbohydrate in the periphery would be increased. Many authors, however, have been unable to find unequivocal evidence of such increased peripheral action of the extra endogenous insulin presumably secreted in response to the sulfonylureas. This is not in complete accord with the expectations.

The β-cytotropic hypothesis, nevertheless, has gained increased support by experiments recently published. Craig, Molzahn, Woodward, and Miller[2] compared the actions of tolbutamide and insulin when injected into the brachial artery on the arteriovenous difference of the forearm. In six nondiabetic subjects they failed to show any effect of tolbutamide on the arteriovenous blood glucose difference. In contrast, insulin in small doses gave highly significant effects. The authors conclude that the data clearly indicate "that under the conditions of these experiments tolbutamide was exerting its blood glucose lowering effect at a nonperipheral site."

A second recent paper is that of Madison and Unger.[3] They compared the effects of glucagon-free insulin injected into the portal vein with that following injection into a peripheral vein. They supposed that "insulin secreted into the portal vein, because it passes directly into the liver, may have a metabolic effect different from insulin administered peripherally." In eight dogs under controlled conditions they determined the blood arteriovenous glucose difference as a measure of peripheral glucose utilization. This was significantly diminished when the same amount of insulin was injected endoportally as compared with peripheral injection. In other words, on the basis of these results it would be possible to suppose that, if the endogenous insulin secreted in response to arylsulfonylurea traversed the liver first, its peripheral action might be abolished or reduced to levels which escape quantitation. The conclusions have obvious significance in the consideration of the β-cytotropic action of these oral hypoglycemic agents.

The paper of Jacobs, Reichard, Goodman, Friedman, and Weinhouse[4] adds further supportive evidence to this hypothesis. In forty diabetic and nondiabetic patients in the postabsorptive state they contrasted the effects of intravenous injections of insulin and tolbutamide on the specific radioactivity of blood glucose after a priming dose of this isotopic sugar. From the levels of blood sugar and its specific radioactivity they calculated: (1) the rate of entry of glucose from the liver into the blood, and (2) its peripheral rate of utilization. In the case of insulin they found, concomitant with the onset of hypoglycemia, that the specific activity of the blood glucose remained constant. This plateau of glucose specific activity is the result of the suppression of glucose output by the liver. In addition, the calculated rate of glucose utilization by the periphery was significantly increased. The same type of data was obtained following the intravenous injection of tolbutamide. In this instance the plateau of specific activity of blood glucose associated with the onset of hypoglycemia also was found as in the case of insulin. Again this evidence indicates a suppression of entry of glucose from the liver into the blood. However, the data indicated no increase in peripheral glucose utilization, a result which is in sharp contrast with that following insulin injection. They concluded "that the lack of influence of tolbutamide on peripheral blood utilization . . . coupled with the virtual certainty that this drug requires insulin for its hypoglycemic action suggests to us that the physiological action of the insulin secreted by the pancreas may also be exerted principally or exclusively on the hepatic glucose output." The validity of these conclusions was strengthened by further observations of these

authors when insulin was given subcutaneously. In this instance, in contrast to intravenous injection, the slow absorption limits the concentration of insulin in the blood to low levels. In consequence, the results obtained imitated those found following the intravenous injection of tolbutamide, namely, a suppression of glucose entry from the liver into the blood stream shown by plateauing of the specific activity of the blood glucose but no peripheral increase of glucose utilization. Presumably as in the administration of tolbutamide, the concentration of insulin in the blood is increased but its action is limited to the liver.

These conclusions, in agreement with those of the other two papers, are in accord with the β-cytotropic hypothesis of the action of the arylsulfonylureas.

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STRESS, CORTICOIDS AND DIABETES

There is abundant clinical evidence that the diabetic patient reacts to severe stress with an aggravation of his disease, often as serious as ketoacidosis and coma. Such stressful experiences may be intercurrent diseases as severe infections and high fever, cardiovascular accidents and shock, or severe trauma, surgical procedures, fractures and burns, and also severe and sudden emotional disturbances. The nondiabetic likewise develops not infrequently under such conditions similar but less marked metabolic changes, characterized among others by hyperglycemia, glycosuria and ketonuria.[1,2,3,4] This phenomenon therefore seems to be neither specific for diabetes mellitus nor for any of the precipitating conditions, but rather to represent part of the nonspecific stress reaction. It is exaggerated in the diabetic only because it is here superimposed upon a previously impaired carbohydrate metabolism.

It is generally assumed that the widespread nonspecific metabolic changes attending stress are largely due to the function of the hormones of the anterior pituitary and the adrenal, which permit adaptation of the body homeostasis to emergency situations. It is as yet uncertain whether this adaptation mechanism requires hyperfunction of the adrenal medulla (Cannon's emergency release), or of the anterior pituitary and adrenal cortex (Selye's adaptation syndrome), or whether normal function of these endocrine glands simply plays a "permissive role" (Ingle).

Whatever the specific role of these hormones may be in the stress and adaptation syndrome, there is ample clinical and experimental proof that they aggravate diabetes mellitus. They affect carbohydrate metabolism by interfering with the action of insulin, by augmenting gluconeogenesis through protein breakdown, and by impairing glucose utilization. ACTH, growth hormone or cortisone and related corticosteroids are well known to induce experimental "steroid" diabetes. Pathological hyperfunction of the pituitary or the adrenals, as in acromegaly, Cushing's disease or pheochromocytoma are frequently accompanied by hyperglycemia and glycosuria, and if complicating diabetes mellitus, aggravate the disease.

Evidence of steroid hyperactivity can also be demonstrated in the diabetic under the stress of either too much or too little insulin. Insulin hypoglycemia[5] as well as withholding of insulin and diabetic acidosis[6] are accompanied by increased urinary output of 11-oxysteroids and 17-ketosteroids, by decrease in circulating eosinophils and by increased urinary excretion of potassium. Re-establishment of blood sugar homeostasis or renewed control of the diabetes abolishes these signs of the stress reaction. The frequently unusually high insulin requirement in diabetic acidosis also is thought to be due, at least in part, to increased steroid activity.[7,8]

Insulin deficiency and steroid hyperactivity, one precipitating the other, appear to be links of a vicious circle in the stress reaction of the diabetic. Whether insulin deficiency is the cause or the sequel of increased steroid activity, the diabetic organism becomes unable to cope with the increased gluconeogenesis attending stress. Adaptation fails and aggravation of the disease occurs. If adequate doses of insulin are supplied, adaptation to the emergency situation is made possible, steroid hyperactivity ceases and the derangement of carbohydrate metabolism returns to its previous degree of impairment.

Thus the stress-induced aggravation of diabetes mellitus, if properly attended to, is as transitory and reversible as the stress syndrome in general. Seldom is the increased insulin requirement permanent.

The recognition that manifest diabetes mellitus can be aggravated by extrapancreatic, functional and environmental factors, leads by necessity to the question of whether these factors can also be operative in the causation of the disease. From a theoretical point of view, one should expect that a disease aggravating factor, if severe enough, may become a disease precipitating or causing factor. The role of environmental influence and of stress is relatively easy to determine where the etiological mechanisms are known and where the onset of the disease is sudden and readily recognizable, as in infections or vascular accidents. In diabetes mellitus, however, we deal with an hereditary or genetic predisposition, and moreover, with a disease which usually has an inconspicuous and insidious onset. Stressful life experiences are common. With some imagination one will find in each diabetic history, coinciding with the first manifestation of the disease, an event which could be described as a causative stress. But a critical analysis will often show that similar events occur as frequently in the history of healthy people and of nondiabetic patients. Many of the case reports of stress-induced or "traumatic" diabetes have thus been unconvincing.[9,10] The lack of objective means to measure and evaluate the impact of a given event on a given individual at a given time has therefore forced most observers to deny any causal relationship between stress or trauma and diabetes mellitus. In their opinion the diabetic predisposition is the main determining factor for the manifestation of the disease, no matter how late in life and under what conditions this may occur. (Environmental influences, particularly overeating and obesity, are accepted as accelerating the manifestation but are not thought to play a role as primary etiological factors.) Aside from the rare instance of surgical diabetes after total pancreatectomy, they are willing to grant only one exception: if the trauma is exerted on the site of the disease, e.g., the pancreas, and if it is severe enough to cause destruction of this organ or of essential parts of it.[11,12] It is evident that such trauma will rarely be survived. The concept of these authors finds strong support in statistical data: that the incidence of diabetes did not increase in the war-stricken countries of Europe during World Wars I and II in spite of the fact that physical and emotional stresses were undoubtedly more frequent and more severe than in peace time;[12] that no increased incidence of diabetes is reported from troops in front lines,[12,20] and that the incidence of diabetes all over the world is rather constant, increasing only with the increase of age of the population and the greater longevity of the diabetic because of better management of the disease. Joslin[13] thus has emphasized recently that he has not found one case of "traumatically induced diabetes" among the 49,000 diabetics under observation in his clinic during the past fifty years. Thomson[14] in Scandinavia came to a similar conclusion in an analysis of trauma and carbohydrate metabolism with special reference to the existence of traumatic diabetes. Similarly, Reed[15,16] states on the basis of his great experience and an exhaustive review of the literature that "trauma and the stressful situations frequently attendant thereto do not cause diabetes mellitus in man."

Other authors, however, without denying the factual data just presented, feel that the evidence is still inconclusive and that the increasing knowledge about extrapancreatic and functional factors affecting blood sugar homeostasis and diabetes strengthens rather than weakens the concept of a stress-induced or traumatic diabetes, rare as this condition may be. They hold that the generalized metabolic effects of trauma as a stress deserve as serious consideration as its localized effects and the resulting local injury. Moreover, the predisposition for the disease, though always present, may not always have the same weight as an etiological factor. Depisch[17] suggests that the "anlage" or inadequacy of the islet cell system may be solely responsible for the manifestation of the disease in childhood, but that the degree and duration of environmental factors gain increasing etiological significance with advancing age. Thus he states that in an aged patient who develops diabetes in connection with a serious illness, as pneumonia, fracture or cerebral hemorrhage, the "stressor" appears to have greater etiologic importance than the "anlage." Similarly, Glatzel[18] reasons that the manifestation of a hereditary disease depends both on the probability of penetration (penetrance-probability) of the "anlage" and on environmental influences, as overnutrition, hormonal factors, puberty and menopause, infections, atherosclerosis, etc. The penetration-probability of diabetes is relatively small. Not all "prediabetics" or siblings of diabetic patients develop the disease. Environmental influences, if severe and long lasting enough, may therefore deserve serious consideration as etiologic factors particularly when the disease manifests itself in close time relationship. In such instances, which may be rare, Glatzel would not even use the term of aggravation since no "grave" condition existed before, but rather precipitation or causation. In line with these views is the ob-

servation of Walker[19] who found in an analysis of 200 consecutive diabetic patients a relatively high incidence of stressful experiences coinciding with the onset of the disease, particularly among the older age group. She concludes, therefore, that stress, though never the sole etiologic agent may have an additive or precipitating effect. Gendel's and Benjamin's[20] finding of severe stress or injury in combat coinciding with the manifestation of diabetes in twelve out of forty-four diabetic soldiers admitted to a general hospital in World War II may have greater significance than that given by the authors themselves. They disregarded in these instances any traumatic origin because the disease did not improve with the relief of the stress situation. Here belong also the cases of persistent diabetes following myocardial infarction in patients who had previously been entirely free of the disease, as reported by Levitt, Handelsman and DiGregorio,[21] the case reports of Kauvar and Goldner,[22] of Piazza,[23] of Gurling and associates,[24] the occasional diabetes attending hyperthyroidism, the permanent diabetes developing in multiparous mothers who have shown bouts of transitory glycosuria during earlier pregnancies,[25] and probably also the diabetes developing in Cushing's disease, acromegaly and pheochromocytoma.[26] These and other such instances may be nothing more than exceptions to the rule. But as exceptions, they demonstrate the occurrence of traumatic or stress-induced diabetes. They cannot be invalidated by the facts that the vast majority of diabetes is not caused by trauma and that trauma in the vast majority of cases does not cause diabetes.

Morbidity statistics are of little help in the decision whether in an individual case the stress reaction turned health or a hidden diabetes into manifest disease. They deal with disease incidence not with time and mode of manifestation. Only careful case analysis of the specific aspects of an individual case with proper consideration of the past history, the severity, duration of the stress situation and its proximity to the manifestation of the diabetes, can ascertain the probability of a causal relationship. Lommel[27] cautions against the employment of statistics as means to minimize the importance of such exceptions, when he states that statistics are misused when employed to contrast mass data with unusual and rare situations. Statistics should evaluate comparable situations. Even the observations during World Wars I and II may not rule out with certainty the role of emotional or physical stress as etiological factors of diabetes. These were years of increased stress but also hunger years, and more remarkable than the absence of a rise in incidence in diabetes may be the actual decrease which occurred. This may well be the result of the nutritional restriction. Certainly stress cannot account for it, unless mass experiences of stress differ from the individual experience.

Great as the difference seems to be between those who are willing to grant stress its disease-aggravating effect but deny its disease-causing role and those who accept both, there seems to be common ground for both groups. As practicing physicians, both agree that the potential as well as the manifest diabetic should avoid severe stress. Both are aware of the serious role of overeating and obesity and use the slogan of diabetes being the punishment for obesity. Obesity certainly is an environmental factor. One also must realize that for practical purposes the manifestation and not the anlage make the patient aware of his disease and impose upon him the health limitations of which he appeared entirely free. A genetic predisposition is not yet a disease. While in diabetes the progress from anlage to manifest disease is commonly slow and inconspicuous, it is occasionally sudden, and highlighted by a severe stressful experience. Not knowing all the factors which cause the disease and only beginning to understand the metabolic implications of stress, can we deny its possible etiologic impact in such instances?

The judge when in doubt decides "in dubio pro reo" (*in doubt, favor the accused*). The physician may have to resort to this time-honored sentence too, particularly at times when medical knowledge undergoes rapid changes as in ours.

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The Russian histologist S. G. Chassovnikov deserves notice as an early student of the islets of Langerhans. He was born in Saratov, a town on the Volga River, on Sept. 23, 1871. After preparatory school he attended the University of Moscow as a medical student and was graduated with honors and a master's degree in medicine in 1895. In 1896, Chassovnikov moved to Warsaw, where he became an assistant to Professor Vollosov at that city's Histological Institute.

He remained in Warsaw until 1912 and during that time revealed his interest in the pancreas. A preliminary report of his research on the excretory parenchyma and islands of Langerhans appeared in *Travaux de la Société des Naturalistes de Varsovie* in 1898. The research was described in detail in his thesis, which was written in Russian and published in Warsaw in 1900. Its title was "Concerning the Morphology and the Functional Changes of the Cells of the Pancreas."

Chassovnikov observed the fine granules in the islet cells. He also noticed the transitional forms between parenchymal cells and islet cells and considered the possibility that the islands were composed of exhausted glandular cells. Work published by Schulze in 1900 and Sobolev in 1900 and 1902 stimulated him to further research. They had noticed that, though the excretory parenchyma was destroyed after ligation of the excretory duct of the pancreas, the islands remained. When diabetes did not follow, they attributed this to the presence of the islands.

Chassovnikov ligated the duct of Wirsung in fourteen rabbits, and observed glucosuria in none of them. He also examined the pancreas at intervals ranging from two to seventy-five days after the ligation. He could find no clear distinction between the islands and the excretory parenchyma in formalin-fixed tissue, and he turned to Hermann's fluid as fixative and stained paraffin sections with safranine and methylene b'ue. In 1898 he had observed that island cell granules exhibited a differing affinity for these dyes. In most cells the granules absorbed methylene blue; in others, especially at the periphery of the island cells, they absorbed safranine. Chassovnikov also observed red granular cells

among the parenchymal cells. In his published research he illustrated these experiments with his own beautiful color drawings. Today we call the red-colored cells, alpha cells and the green, beta cells.

In 1906 Chassovnikov described two cell types in the islands of the rabbit which he could stain differentially in a single section. A year later Lane, unaware of Chassovnikov's publication, described both cell types in the guinea pig at Bensley's laboratory. Lane used various fixatives, stained with neutral gentian violet, and therefore did not observe the two cell types in a single section. He talked of A and B cells and suspected that they would excrete a different matter. In 1911, Bensley improved Lane's method, wrote of A and B cells and succeeded in staining both differentially in one section.

Chassovnikov frequently observed proliferation of the connective tissue through the islands the third week after ligating the duct. In this way the islands were divided into small groups, each always containing both cell types. The cells did not show any sign of degeneration, thus disproving the hypothesis that island cells were actually exhausted glandular cells. Because the islands persisted and diabetes did not appear after ligation, Chassovnikov, together with Schulze and Sobolev, concluded that the islands were organs of internal secretion which regulate carbohydrate metabolism. Chassovnikov came to this conclusion and surmised that there was a missing link in the pattern of experimental diabetes, i.e., that diabetes would appear after extirpation of the islands persisting in the atrophic pancreas. However, he was unable to prove this final point, as no more research could be conducted at Russian universities. He also had to give up his intention to do histological research on the island cells after intravenous administration of glucose. He was convinced that this was the only way to examine the function of the islands more closely, because the pancreas of people who died of diabetes was unusable as a result of postmortem changes. Furthermore, he considered it possible that the liver as well as the islands played a role in diabetes.

In 1911 Chassovnikov entered in competition for a professorship at Tomsk. He was elected, but the Ministry refused his nomination on grounds that he lacked pedagogical experience. Nevertheless, in 1912 he was appointed Extraordinary Professor in Histology and Embryology at Tomsk and this was changed four years later into a regular professorship. In October of 1913 he was elected Secretary of the medical faculty, from which position he had to resign three years later because of tuberculosis. Chassovnikov then applied for a professorship in southern Russia, hoping that the change in climate would cure him. In 1917 he was chosen Professor in Histology and Embryology at Kiev, but the civil war made the move impossible. On Sept. 26, 1926, Chassovnikov died of tuberculosis at the age of almost forty-nine.

Chassovnikov had great scientific gifts and enormous energy. He wrote and spoke fluent English, French and German. While still a student, he translated a German textbook into Russian. He was an excellent lecturer and though an uncommunicative person with a morose manner, he was very well liked by students for his simplicity and strong sense of justice. His primary interest was cytophysiology, and the endocrine glands especially fascinated him. He believed that a perfect histological technic was fundamental for scientific research and therefore made all preparations himself. In this respect he demanded very high standards of himself and his students. In the Histological Laboratory at Tomsk a great collection of preparations made by Chassovnikov can be found. They are striking for the aesthetic care with which they were prepared. He wrote approximately twenty articles, of which some were published for government records a year after his death. A son of the prematurely stricken scientist followed in his father's footsteps and also became professor of histology in Russia.

ACKNOWLEDGMENT

Because the archives of the Warsaw University were removed to Rostov in 1915 and destroyed in the Second World War, the author was unable to acquaint herself with Chassovnikov's publication of 1898.

The author extends her thanks to the directors of the Histological Laboratory of the University in Tomsk, the University Library in Warsaw and the Institute of Medical History in Moscow for their cooperation in obtaining a portrait of Chassovnikov, a microfilm of his thesis and biographical data.

BIBLIOGRAPHY

Tschassownikow, S.: Über die histologischen Veränderungen der Bauchspeicheldrüse nach Unterbindung des Ausführungsganges, Zur Frage über den Bau und die Bedeutung der Langerhansschen Inseln. Arch. mikrosk. Anat. u. Entw. Gesch. 67: 758, 1906.

EDITOR'S NOTE: Illness has prevented Dr. van Beek from completing or proofreading the English translation of her biographical sketch of this little known student of the pancreas and the islets of Langerhans. The Editors are certain that all readers will be grateful to Dr. van Beek for her effort in drawing attention to the historically important contribution of Chassovnikov.

BOOK REVIEWS

CHEMISTRY OF LIPIDES AS RELATED TO ATHEROSCLEROSIS. *A Symposium compiled and edited by Irvine H. Page, M.D. $8.50, pp. 342, Charles C Thomas, Springfield, Illinois, 1958.*

This stimulating book contains the transcript of a Symposium held at the Cleveland Clinic under the auspices of the National Heart Institute in May, 1957. Eighteen of the participants presented formal papers dealing with three areas of lipid biochemistry: chemistry and distribution of lipids; absorption and transport of lipids; and cellular metabolism of lipids. In the Introductory Talk, Dr. Page points out that the Symposium does not deal with "the important implications of the 'other than fat' mechanisms which . . . are involved in atherogenesis." In fact, the reader of this book will most probably come away with the impression that the precise role of lipids in atherogenesis likewise still remains to be established. This by no means detracts from the value of this book to all investigators interested in the nutritional and biochemical aspects of lipid metabolism.

The papers presented, ranging from the "Behavior of Unsaturated Acids on the Gas-Liquid Chromatogram," by A. T. James, to the "Enzymatic Synthesis of Phospholipids and Triglycerides," by E. P. Kennedy, are generally of high quality and represent stimulating reviews of recent research (up to May, 1957). Investigators studying the metabolic pattern of the diabetic organism will be interested in the chapters dealing with the "Transport of Non-Esterified Fatty Acids in Plasma," by V. P. Dole, and the "Biosynthesis of Fatty Acids in Cell-Free Preparations of Mammalian Liver," by R. G. Langdon. They provide further evidence of the close interrelationship of fatty acid and carbohydrate metabolism.

HORMONAL REGULATION OF ENERGY METABOLISM. *Compiled and edited by Laurance W. Kinsell, M.D. $5.25, pp. 242. Charles C Thomas, Springfield, Illinois, 1957.*

This volume represents the formal presentations and discussions of a conference sponsored by a group that term themselves The Metabolic Conference. The meeting was arranged on the premise that a need existed for a conference on the hormonal regulation of energy metabolism.

Perhaps the major features of this book are the relative brevity of most of the formal presentations and the extensiveness of the discussion, in which many worth-while ideas are expressed. The formal papers are listed as follows:

1. "Certain Aspects of Hormonal Regulation of Carbohydrate Metabolism," by DeWitt Stetten, Jr., M.D.
2. "Hormonal Regulation of Enzymatic Activity," by Henry A. Lardy, Ph.D.
3. "The Anterior Pituitary in Relation to Energy Metabolism," by Bernardo Houssay, M.D.
4. "The Mechanism of the Influence of Pituitary Growth Hormone on Metabolism," by P. J. Randle, M.D., and F. G. Young, D.Sc.
5. "The Thyroid in Relation to Energy Metabolism," by Jack Gross, M.D.
6. "Insulin—Reminiscences," by Charles H. Best, M.D.
7. "Diabetes and the Insulin Problem," by William C. Stadie, M.D.
8. "The Adrenal Cortex and Energy Metabolism," by E. B. Astwood, M.D.

In addition to the above listed presentations, Dr. Elliott P. Joslin has prepared the opening remarks of this volume, which are presented under the title, "The Road Ahead." It is very fitting that this volume should be introduced by Dr. Joslin.

It is to be regretted that relatively few of the formal presentations include a bibliography and that the volume itself has no index. It is therefore difficult, in many instances, for the reader to ascertain the basis for some of the statements made both in the presentations themselves and in the discussion.

It is certain that investigators of metabolism will find this volume full of interesting and provocative comments.

The volume could have been increased in value and made more authoritative by including consideration of previous publications in the vast field of intermediary metabolism.

DIABETES AS A WAY OF LIFE. *By T. S. Danowski, M.D. $3.50, pp. 177, Coward-McCann, Inc., New York, 1958.*

Dr. Danowski's entrance into the field of lay literature results in a veritable miniature textbook written in language that the patient can understand. In the author's usually complete and precise manner all phases of diabetes are discussed. These include signs and symptoms, diet, insulin, indications for and against stopping insulin including the use of the oral hypoglycemic substances, factors rendering diabetes more severe, hypoglycemia, social problems, infections and surgery, acidosis and pregnancy. In an attempt to be reassuring to the patient, the chronic complications such as atherosclerosis and retinopathy have been given a very gentle touch and are actually not stressed.

The general text is rounded out by an excellent, well-thought-out question-and-answer check list for diabetics and a complete and accurate glossary. This is followed by a very good appendix which includes information and clear instructions concerning the testing for urinary sugar by the different methods in common use today as well as a description of the various insulins used at the present time.

A unique and extremely useful section of the appendix lists the names and addresses of the Affiliates of the American Diabetes Association.

It seems too bad that Dr. Danowski did not carry out this ADA theme in relation to diets as well. He accurately reproduces the *Meal Planning with Exchange Lists* and gives proper credit for this system, but then makes mention and uses as his illustrative diets various tables obtainable without charge from two of the insulin manufacturers. It would seem more advantageous to mention and make use of the diet lists available through the American Diabetes Association.

In general this is an excellent book on diabetes for the lay person. However, it is too complete and painstaking for the overly curious patient who might tend to misinterpret certain aspects, such as the discussion of the role of other endocrine glands. Conversely, the superficial reader might be better off reading a less profound primer on diabetes. For example, some of the charts intermingled with the text are similar to those found in medical textbooks, and the reviewer fears that they might confuse rather than enlighten some readers. On the other hand, for the intelligent, well-adjusted and alert diabetic who seeks accurate and well-rounded knowledge about his disease, this volume should prove to be superb.

ABSTRACTS

Alagna, G.: MUCOPROTEINURIA IN DIABETIC RETINOPATHY. Arch. di ottal. *61*:99-109, March-April 1957. (Abstracted in Am. J. Ophth. *44*:720, November 1957.)

Patients with diabetic retinopathy show an increase of muco-proteins in blood and urine, the author states. This finding suggests that the retinal lesions are part of a general mesenchymal disturbance, expressed by the increased excretion of muco-proteins. (Italian)

Anonymous: MURDER BY INSULIN. Pharmaceutical J. *179*:486, Dec. 21, 1957.

After a trial at Leeds Assizes on Dec. 13, 1957, as a result of which a charge nurse was found guilty of the murder of his wife by injecting her with insulin, the judge paid tribute to those responsible for the scientific research that went into the case. A whole new field of investigation into the extraction of insulin products from human tissue had to be undertaken. Boots Pure Drug Co., Ltd., was consulted in some of the work. It was the first case of murder by insulin.

Ansell, G. B.; and Dohmen, H. (Med. Res. Council, Neuropsychiatric Res. Unit, Whitchurch Hosp., Cardiff, Wales; Physiologisch Chemisches Inst. der Universität, Köln, Germany): THE METABOLISM OF INDIVIDUAL PHOSPHOLIPIDS IN THE RAT BRAIN DURING HYPOGLYCEMIA, ANAESTHESIA AND CONVULSIONS. J. Neurochem. *2*:1-10, 1957.

The uptake of phosphorus into the individual phospholipids of rat brain in vivo was studied and considerable variation in turnover observed. The rate of uptake decreased in this order: diphosphoinositide > phosphatidyl choline > phosphatidyl ethanolamine > phosphatidyl serine. In the thiopentone anesthesia the incorporation of phosphorus into phosphatidyl choline was reduced to 25 per cent and into phosphatidyl ethanolamine to 33 per cent of the normal. In insulin hypoglycemia the decreases were to 40 per cent and 72 per cent respectively.

Antoniades, Harry N.; Beigelman, Paul M.; Pennell, Robert B.; Thorn, George W.; and Oncley, John L. (Dept. of Biophysical Chem., Harvard Univ., Cambridge, Mass., & Dept. of Med., Harvard Med. Sch., and Peter Bent Brigham Hosp., Boston, Mass.): INSULIN-LIKE ACTIVITY OF HUMAN PLASMA CONSTITUENTS. III. ELUTION OF INSULIN-LIKE ACTIVITY FROM CATIONIC EXCHANGE RESINS. Metabolism 7:266-68, May 1958.

Elution of insulin-like activity from cationic exchange resins from which the activity had been absorbed from human plasma was achieved by treatment of the resin with citrate at *p*H 3.0 for twenty-four hours. Crystalline insulin was not absorbed by cationic exchange resins.

Aschner, B. M.; and Post, R. H. (Columbia Univ., New York, N.Y.): MODERN THERAPY AND HEREDITARY DISEASES. Acta genet. et Statistica med. *6*:362-69, 1956-1957.

Diabetes is discussed from the viewpoint of population genetics as one of several diseases under genetic control that are responsive to modern therapy. An estimate is made of the rate of increase in the United States of the frequencies of the genes producing (under certain conditions) diabetes, as a result of this therapy. Variation in the penetrance of the diabetes-producing gene or genes among different races, economies and cultural epochs of prehistory is discussed, as background for tentative answers to the question of why a genetically controlled disease should have as high a frequency as diabetes when natural selection is operating against it.

Beigelman, Paul M.; and Antoniades, Harry N. (Dept. of Med., Univ. of Southern Calif. Med. Sch.; Los Angeles County Hosp., Los Angeles, Calif.; Protein Foundation, Inc., Jamaica Plain, Mass.; Dept. of Gynecology, Harvard Med. Sch., Boston, Mass.): INSULIN-LIKE ACTIVITY OF HUMAN PLASMA CONSTITUENTS. IV. INSULIN LEVELS OF NORMAL HUMAN SERUM AND PLASMA. Metabolism 7:269-73, May 1958.

Insulin-like activity of pooled human serum is estimated to be 0.25 to 1.4 milliunits/ml. by two different methods of assay. The authors believe that the marked discrepancies detectable in the serum and plasma insulin values reported by different investigators are probably the result of multiple insulin-like and anti-insulin factors.

Bell, D. J. (Agricultural Research Council, Poultry Research Centre, Edinburgh, Scotland): THE DISTRIBUTION OF GLUCOSE BETWEEN THE PLASMA WATER AND THE ERYTHROCYTE WATER IN HEN'S BLOOD. Quart. J. Exper. Physiol. *42*:410-16, October 1957.

The blood erythrocyte volume (EV) was measured in hens; it is highest in nonlaying birds. The water contents of the whole blood and plasma have been determined, and that of the erythrocytes calculated from them. Most (95 per cent) of the blood glucose is in the plasma water. The remaining 5 per cent of the blood glucose is present in the water of the erythrocytes; its concentration there is only one fifth to one sixth of its concentration in the plasma water. Changes noted by others in blood sugar levels of hens treated with substances showing sex-hormone activity are believed to result from alterations in EV.

A parallel is noted between the fall in EV during human pregnancy and during ovulation in the hen.

Berg, Max; and Levinson, Samuel A. (Depts. of Pathology and Internal Med., Univ. of Illinois Coll. of Med. and Louis A. Weiss Mem. Hosp., Chicago, Ill.): HYPERGLYCEMIA INDUCED IN DOGS BY CLOSTRIDIUM PERFRINGENS TOXIN. A.M.A. Arch. Path. *64*:633-42, December 1957.

Lethal doses of C. perfringens toxin injected intramuscularly into the hindlimbs of dogs, which were then massaged, produced a hepatic glycogenolysis with hyperglycemia and without glucosuria. There was also a concomitant increase in serum inorganic phosphorus. Injections of arginine, glycine, methionine and dextrose prior to the injection of the toxin prevented the hyperglycemia. Prior injections of saline solution did not affect the hyperglycemia. Dihydroergotamine administered prior to the C. perfringens toxin may result in a delay of hepatic glycogen breakdown. In pancreatectomy plus toxin there was observed a more pronounced glycogen breakdown. Dihydroergotamine injected prior to toxin is accompanied by a delayed hyperglycemia. Pancreatectomy plus toxin resulted in a more pronounced elevation of blood glucose and inorganic phosphorus.

Bibergeil, H. (Karlsburg, Kr. Greifswald, East Germany): CLINICAL EXPERIENCES WITH INSULIN-LENTE. Deutsche med. Wchnschr. *83*:761-63; *83*:807-13, April 25, & May 2, 1958.

Experiences with Lente insulin at the Diabetes Home Garz and Karlsburg are reported. Of 138 hospitalized patients with diabetes of varying degrees of severity, 115 could be controlled satisfactorily with Lente insulin. The required daily dose given in one injection was usually equal to the total dose of regular insulin given in several injections during the day. During the period of change-over a few units of regular insulin were added to the long-lasting preparation. Replacement with Lente insulin was easier the lower the daily requirement. For the whole group the average daily dose was 29 units, the maximal dose 68 units. The only side effect was a local reaction in one patient. Juvenile diabetes is generally rather difficult to control with long-lasting insulin preparations, yet two thirds of the author's cases with juvenile diabetes responded well to the single daily injection of Lente insulin. During complications (infections, operations, etc.) the patients may have to be put back on regular insulin. The author advises against interrupted insulin treatment on intermittent days. (German)

Blau, Seymour H. (Jersey City, N.J.): DIABETIC ULCERATION WITH BUERGER'S DISEASE—CASE REPORT. J. Nat. A. Chiropodists. *47*:68-71, February 1957.

Arlidin (Nylidrin Hydrochloride N.N.R.), a new drug with unique pharmacologic properties, has been prescribed for conditions involving impaired circulation to the foot. Used in proper dosage, Arlidin is a valuable therapeutic agent for the podiatrist for improving circulation in the foot in patients with occlusive vascular disease, thus contributing to the healing of ischemic ulcerations.

Bonting, Sjoerd L.; Pollak, Victor E.; Muehrcke, Robert C.; and Kark, Robert M. (Dept. of Biol. Chem. and Med., Univ. of Illinois Coll. of Med., and Depts. of Med., Presbyterian-St. Luke's Hosp., Cook County Hosp., and Res. and Educational Hosps., Chicago, Ill.): QUANTITATIVE HISTOCHEMISTRY OF THE NEPHRON. Science *127*:1342-43, June 6, 1958.

The authors report a technic to identify and dissect out various portions of the nephron by adapting to the analysis of renal tissue the ultramicrotechnics developed by Lowry for analysis of brain tissue.

Burns, Thomas W. (University of Missouri Medical Center, Columbia, Mo.): ORAL ANTIDIABETIC COMPOUNDS. Missouri Med. 55:475-77, May 1958.

A brief discussion of the chemical nature, history, and possible mechanism of action of the sulfonylurea compounds is given. The author gives his regimen for selection of patients for use of the oral drug at the University of Missouri Clinics.

Chow, Bacon F.; and Stone, Howard H. (Dept. of Biochemistry, Sch. of Hygiene and Public Health of Johns Hopkins University, and Wilmer Ophthalmological Inst. of Johns Hopkins University and Hosp., Baltimore, Md.): THE RELATIONSHIP OF VITAMIN B_{12} TO CARBOHYDRATE METABOLISM AND DIABETES MELLITUS. Am. J. Clin. Nutrition 5:431-39, July-August 1957.

The dominant role that vitamin B_{12} plays in the utilization of carbohydrates is now well established. This article summarizes this aspect of the metabolic role of the vitamin, the interrelationship between Vitamin B_{12} absorption and endocrine function, and finally the relationship of vitamin B_{12} to diabetes mellitus. Some significant findings are as follows:

Prolonged injection of cortisone or corticotropin into normal rats will bring about hyperglycemia correctable by the injection of vitamin B_{12}. Another effect of cortisone administration is elevation of the vitamin B_{12} serum level. It may be that cortisone, like carbon tetrachloride, causes injury to liver with subsequent release of vitamin B_{12}. The thyroid hormone and vitamin B_{12} are intimately related in the fact that thyroidectomy in rats prevented the absorption of orally administered radioactive vitamin B_{12}. The normal absorption could be restored in such animals by incorporating desiccated thyroid in the diet. Studies are presented involving retinopathic and nonretinopathic diabetics as well as nondiabetics in relation to B_{12} metabolism. These results may be summarized as follows:

After an intramuscular test dose of vitamin B_{12}, diabetic subjects with retinopathy excrete significantly more of the vitamin than the nondiabetics, while diabetics without retinopathy excrete less than the nondiabetics. In line with the oft-tried therapy of diabetic retinopathy with testosterone, the authors point out that the administration of testosterone to diabetics with retinopathy decreases the urinary excretion of a test dose of vitamin B_{12}. There is an elevation of vitamin B_{12} serum levels in patients with retinopathy. In absorption studies of orally administered vitamin B_{12} the authors' data tend to show that diabetics without retinopathy absorbed less of the orally administered vitamin B_{12} than nondiabetics. Other hormonal studies are cited such as the fact that diabetic patients with retinopathy excrete a significantly larger amount of 17-hydroxycorticosteroids than diabetics without retinopathy. It was found that ACTH or cortisone causes hyperglycemia, destruction of vitamin B_{12}-binding substances in muscles as well as an elevation of the vitamin B_{12} serum level. Transference of these phenomena may be difficult because of the significantly different vitamin B_{12} levels in different animal species.

Cutforth, Robert H.; and Powell, M. E. A. (King's Coll. Hosp. Med. Sch., London, England): DIABETES MELLITUS CAUSED BY SECONDARY CARCINOMA OF THE PANCREAS. Brit. M. J. *1*:205, Jan. 25, 1958.

The authors report a case of infiltration of the pancreas by secondary carcinoma, and resulting diabetes mellitus: The patient had a family history of diabetes mellitus, and the possibility of hereditary factors could not be ruled out in this case. The rarity of diabetes mellitus associated with secondary infiltration of the pancreas may be explained on the basis that patients succumb to the effects of the neoplasm before sufficient damage has been done to the islet tissue to produce diabetes.

Dekaban, Anatole S.; and Magee, Kenneth R. (National Inst. of Neurological Diseases and Blindness, NIH, Bethesda, Md.; University Hosp., University of Michigan, Ann Arbor, Mich.): OCCURRENCE OF NEUROLOGIC ABNORMALITIES IN INFANTS OF DIABETIC MOTHERS. Neurology *8*:193-200, March 1958.

There is satisfactory evidence that the incidence of fetal death in diabetic mothers is very high. Similarly, the rate of congenital malformations, as estimated from autopsy findings, is greater than in the normal population. It is suggested that the incidence of morbidity due to involvement of the central nervous system in the surviving offspring of diabetics is also higher than in children born to nondiabetic mothers. The chief clinical abnormality in all four surviving infants described in this paper consisted of a severe degree of mental deficiency. Cerebral diplegia was also present in two of the patients. The

fifth patient had pronounced malformations of many organs, particularly of the brain, which were incompatible with life. The nature of the cerebral abnormalities found in infants without prenatal malformations needs further investigation.

Ditzel, Jørn; Sargeant, Lynn; and Hadley, William B. (New England Deaconess Hosp., Joslin Clinic, and Dept. of Med., Harvard University Med. Sch., Boston, Mass.): THE RELATIONSHIP OF ABNORMAL VASCULAR RESPONSES TO RETINOPATHY AND NEPHROPATHY IN DIABETICS. A.M.A. Arch. Int. Med. *101*:912-20, May 1958.

A study of vascular response patterns observed microscopically in the bulbar conjunctiva was made in sixty young diabetics with retinopathy and nephropathy. The significant relationship between small-vessel degeneration in the retina and the kidney and the vascular response patterns observed microscopically in the bulbar conjunctiva of young diabetics is of considerable interest. This association cannot be explained by differences in duration of diabetes or various degrees of immediate control, because these factors are comparable in the groups studied. Since the well-defined pattern deviations may be reversible and are present even in patients with diabetes of short duration, the changes in the pattern are not caused by vascular degeneration. Thus the relationship indicates that the abnormal response patterns of the smaller blood vessels are an important factor in the development of diabetic retinopathy and possible nephropathy.

Dobson, Harold L.; Lipscomb, Harry S.; Greene, James A.; and Engelhardt, Hugo T. (Dept. of Internal Medicine, Baylor University Coll. of Medicine, Diabetic Serv., Jefferson Davis Hosp., Houston, Tex.): SOCIO-ECONOMIC STATUS AND DIABETES MELLITUS. J. Chron. Dis. 7:413-21, May 1958.

The diabetic patient attending a charity clinic presents a multitude of social, economic, and medical factors that tend to make him a much different problem from the diabetic under private care. These charity patients are faced with extremely limited financial resources, are on the average in a lower intelligence group, and are often given rather superficial medical care. All too often, there is less interest in the patient than in his urine or blood sugar. Some of the measures employed to correct the more serious problems are discussed briefly.

Duncan, L. J. P.; MacFarlane, A.; and Robson, J. S. (Univ. Edinburgh, and Royal Infirmary, Edinburgh, Scotland): DIABETIC RETINOPATHY AND NEPHROPATHY IN PANCREATIC DIABETES. Lancet *1*:822-26, April 19, 1958.

The precise relationship between the development of the specific vascular complications of diabetes mellitus in the retinae and of specific nodular glomerulosclerosis to the disorder itself is unknown. A patient is described in whom both these complications occurred and in whom there was overwhelming evidence to support the view that the diabetes arose as a result of pancreatic disease. The significance of this case in relation to present-day views of the etiology of these complications is discussed; it constitutes strong evidence in favor of the view that these complications are the consequence of the metabolic disorder which results from insulin deficiency.

Engel, Frank L. (Depts. of Medicine, Physiol., Director of Div. of Endocrinology, Duke Hosp., Durham, N.C.): THE INFLUENCE OF THE ENDOCRINE GLANDS ON FATTY ACID AND KETONE BODY METABOLISM. Am. J. Clin. Nutrition 5:417-30, July-August 1957.

This article gathers together the many facets of ketonemia with regard to carbohydrate, fat and protein metabolism. Dr. Engel points out that ketone metabolism is an indirect reflection of the metabolism of acetyl Co-A. One of his diagrams pictures acetyl Co-A as the center of intermediary metabolism, thus broadening the scope from which the subject of ketosis and ketone metabolism can be studied. Acetyl Co-A may be derived from carbohydrate metabolism via pyruvate, from fat catabolism by successive beta-oxidation of fatty acids and from protein through various possible pathways. Thereafter, acetyl Co-A may enter the Krebs cycle, may form longer chain fatty acid and eventually neutral fat, phospholipids, etc., and may be an intermediary in the synthesis of cholesterol and steroid hormones as well as purines, heme, porphyrins, and in acetylation reactions. In the clinical consideration of insulin and diabetes he points out that in the absence of insulin, glucose utilization is inhibited, lipogenesis from carbohydrate is reduced (presumably by a specific block in the conversion of acetyl Co-A to fat) and oxidation of glucose by way of the Krebs cycle is depressed. In addition, fat is mobilized to the liver and is catabolized at an accelerated rate. Insulin corrects the basic defect in carbohydrate utilization and fat synthesis and thus overcomes the ketosis, provided adequate carbohydrate is available for metabolism.

The post-hypoglycemic ketosis or ketonemia is also emphasized and is due to an increased hepatic production of ketone bodies. Even such phenomena as increase in nitrogen metabolism following hypoglycemia bear upon the final fat catabolism and production of ketosis. Further facets of metabolism and the effect of endocrine output on carbohydrate metabolism are explored in discussing the adrenal medulla, adrenal cortex and the adenohypophysis. The author points out that neither the adrenal medulla, the adrenal cortex, nor hypophysis is essential to the development of ketosis during fasting or hypoglycemia. Glucocorticoids actually suppress the ketosis resulting from fasting. With respect to the hypophysis the problem is complicated by the question of whether or not there are several different ketogenic pituitary factors. Whatever the answer, they all seem to have one property in common—that is, they also cause a mobilization of fat from the periphery to the liver, thereby providing an increased substrate for ketone production in that organ. The growth hormone also inhibits glucose utilization for fat synthesis and oxidation, stimulates fat catabolism, blocks insulin action and promotes anabolism. All of these factors favor ketone body production.

Fabrykant, Maximilian (Dept. of Med., New York Univ. Post Grad. Med. Sch. & the Univ. Hosp., New York, N.Y.): USE OF ORINASE AS A BASIC ADJUVANT IN MANAGEMENT OF INSULIN-DEPENDENT DIABETES. Metabolism 7:213-21, May 1958.

The author demonstrates the usefulness of Orinase in diabetic patients who are insulin-dependent. There was a 10 to 70 per cent reduction in insulin requirement in the stable and 10 to 50 per cent in the labile diabetic patients. Greater uniformity of control and an increased sense of well-being were also observed. Twenty-eight stable and nine unstable diabetics were benefited whereas therapy failure was experienced by ten other stable and three other labile patients.

The author demonstrates that a short-term use of Orinase may not indicate the beneficial effects which can occur from

more prolonged administration of the drug. It was also shown that in some subjects complete replacement of insulin by Orinase may only be temporary for two or three months. Hence, Orinase loading tests or short-term usage of the drug may be misleading. In six diabetic patients with angina pectoris, striking relief of anginal distress coincided with the use of Orinase.

Fagerberg, Sven-Erik (Med. Dept., Sahlgren's Hosp., Univ. of Gothenburg, Gothenburg, Sweden): STUDIES ON THE PATHOGENESIS OF DIABETIC NEUROPATHY. IV. ANGIOPATHIA DIABETICA VASAE NERVORUM. Acta med. scandinav. *159*: 59-62, Oct. 30, 1957.

A brief reference to the literature on specific vascular changes in diabetes is given. By means of biopsies of the sural nerve, which so far have been performed on about 150 patients —diabetics in different age groups with varying duration and control cases—the author has tried to prove that diabetic neuropathy may be caused by specific angiopathy in the vasa nervorum. The diabetic preparations are compared with those from control patients of about the same age. If the continued investigation shows the same tendency, the author is ready to believe that these results point to angiopathy—specific or both specific and unspecific (neuropathy in elderly persons)—being an important, perhaps even the only, factor in the pathogenesis of diabetic neuropathy.

Freeman, A. G. (Bristol Royal Infirmary, Bristol, England): SYMPTOMS AND CLINICAL ASPECTS OF DIABETES MELLITUS. Brit. M. J. *1*:1149-54, May 17, 1958.

The author reports a study of 300 cases of diabetes mellitus. In 80 per cent of patients the diagnosis was made by the family doctor; in 16 per cent in hospital when advice was sought for symptoms or complications of unrecognized diabetes; and in 4 per cent symptomless glycosuria of diabetic origin was discovered at routine medical examination. At time of diagnosis ketonuria was present in every patient under twenty years, in approximately two thirds of patients in the twenty to thirty-nine age group, and in one third of patients of forty and over. It was present in 25 per cent of patients over sixteen years of age who were obese and in 50 per cent who were not obese. An acute onset of symptoms, occurring within a two-month period, was common among diabetics under forty with ketonuria, while an insidious onset was common among older patients without ketonuria. A comparison was made of the symptoms of the disease in the ketotic and nonketotic cases. One or other of the five classical symptoms of diabetes was present in 95 per cent of cases. As a group they were present in only 24 per cent of patients with ketonuria and in 15 per cent of patients without ketonuria. Loss of weight without previous obesity was a characteristic feature of the ketotic group. A sudden change from an excessive appetite to anorexia was also of considerable diagnostic significance. Loss of appetite often preceded the onset of acute abdominal pain, nausea and vomiting in cases of severe diabetic ketosis. Pruritis vulvae was the only diabetic symptom which occurred more often in the nonketotic group. Visual disturbances, paraesthesias, and peripheral pain did not vary significantly with age. The incidence of heredity was 34.3 per cent. Before the onset of symptoms 45 per cent of patients over sixteen years were 10 per cent or more above standard weight; 43 per cent were of standard weight; and 12 per cent were 10 per cent or more below standard weight. The incidence of marked obesity in diabetic patients over forty was almost twice as great in the presence of hypertension. In only two cases was the normal weight more than 30 per cent below standard. In order to control the disease, insulin was required in 94 per cent of the ketotic group and in 55 per cent of the nonketotic group.

Every patient under sixty years who was underweight on diagnosis required insulin, while 42 per cent of obese diabetics could be controlled by diet alone.

Fritz, Irving B.; Shatton, Jennie; Morton, John V.; and Levine, R. (Dept. of Metabolic and Endocrine Res., Michael Reese Med. Res. Inst., Chicago, Ill.): EFFECTS OF EPINEPHRINE AND INSULIN ON GLUCOSE DISAPPEARANCE IN EVISCERATED DOGS. Am. J. Physiol. *189*:57-62, April 1957.

Epinephrine infusions inhibited glucose disappearance in insulinized eviscerated dogs at both normoglycemic and hyperglycemic blood levels. In contrast, it had no acute effects on glucose disappearance in noninsulinized preparations, regardless of whether the animals were maintained at low or high blood sugar levels. The action of insulin on the final distribution volume of galactose was not affected by the simultaneous infusion of epinephrine. These data, in conjunction with various findings in the literature, were interpreted to signify that the metabolic fate of glucose after the administration of insulin was different from that occurring at hyperglycemic blood levels in the absence of insulin.

Gordon, Maria F. (Inst. of Physiol., Faculty of Medicine, Buenos Aires, Argentina): ACTION OF BZ-55 (CARBUTAMIDE) ON GLUCOSE TOLERANCE, LIVER AND MUSCLE GLYCOGEN OF ADRENALECTOMIZED DOGS. Acta physiol. latinoam. 7:117-23, 1957.

The action of a simultaneous injection of carbutamide (BZ-55) (100 or 200 mg./kg.) and glucose (3 gm./kg.) was studied in adrenalectomized dogs. Glucose tolerance was significantly modified. Ninety minutes after glucose injection, the blood sugar of animals treated with 200 mg./kg. of carbutamide was less than 50 per cent of the blood sugar of the adrenalectomized controls. No significant differences were observed, with respect to muscle and liver glycogen content, between carbutamide-treated animals and control ninety minutes after glucose injection.

Grand Rounds Medical Conference (St. Louis University Sch. of Medicine, St. Louis, Mo.): FATTY LIVER, PANCREATIC FIBROSIS AND MILD DIABETES MELLITUS. Missouri Med. 55: 598-603, 606, June 1958.

The case of a sixty-seven-year-old woman with the finding, surgically, of pancreatic fibrosis and fatty liver coupled with a clinical mild diabetes mellitus is used as the basis of a discussion of the relationship of the pancreatic enzymes and insulin in the control of fatty livers, by Dr. Charles Best, physiologist. He points out that insulin facilitates fat formation in the liver, but without insulin the liver becomes fatty, apparently due to mobilization of depot fat. Diets deficient in choline and methionine also produce fatty livers. Protein deficiency not of the choline or methionine variety also produces a mildly fatty liver.

Pituitary hormone, especially growth hormone, mobilizes fat from the depots to the liver. Protein deficiency alone can produce acinar changes. In the human, however, Dr. Best would not venture to say which comes first, pancreatic enzyme dysfunction or islet cell degeneration.

Gross, Milton: Sexton, Robert F.; and Dorian, Robert I. (Margaret Hague Maternity Hosp., Jersey City, N.J.): DIABETES AND PREGNANCY: I. THE SIGNIFICANCE OF MELITURIA IN PREGNANCY. A PRELIMINARY REPORT. Bull. Margaret Hague Maternity Hosp. 9:211-35, Winter 1956.

This is a preliminary report of a study of the significance of melituria in pregnancy. In approximately half of the urines which gave a positive reaction with Benedict's solution, this was due to true glycosuria (positive Tes-Tape or Clinistix). Ketonuria was found in as many as 10 per cent of the fasting urines examined. The possible causes for glycosuria in pregnancy are discussed. The data collected were analyzed with a view to demonstrating the incidence of those obstetrical factors commonly associated with diabetes or prediabetes in pregnancy. In each factor compared, namely, maternal obesity, large fetal size, abnormal parturition, and ketonuria the incidence is higher in the group in which true glycosuria was found. Although the sample is yet admittedly small, the evidence suggests that the glycosuria of pregnancy may not be as benign as generally considered. Consequently, constant routine examination for urine sugar is a mandatory part of prenatal care. This is now facilitated by the availability of glucose-specific enzyme-impregnated test papers (Tes-Tape and Clinistix), which permit differentiation of true glycosuria from the other nonglucose reducing substances more commonly found in pregnancy urine.

Hasselblatt, A.; and Bludau, W. (Institut für Pharmakologie, Universität Göttingen, Germany): RELATION BETWEEN DOSAGE, SERUM CONCENTRATION AND HYPOGLYCEMIC EFFECT OF N-METHYL-BENZOLSULFONYLUREA AT PROLONGED INTRAVENOUS INFUSIONS. Klin. Wchnschr. 36:157-63, Feb. 15, 1958.

Carbutamide in various concentrations was infused continuously for four hours into normal rabbits. At concentrations and infusion rates of 18 mg./kg./hr., and 36, 72, 144 and 216 mg./kg./hr., the blood sugar levels showed initially gradual decreases, the slope of which varied with the sulfonylurea concentration of the infusate. After two hours, however, the same hypoglycemic levels were reached regardless of concentration, and were maintained for the remaining two hours of the experiment. The serum sulfonylurea levels, on the other hand, differed markedly with the various concentrations and rose gradually during the four hours of the infusion. Appropriate calculations showed that the hypoglycemia was linear with the logarithm of the infusion dosage (mg./kg./hr.), while the sulfonylurea serum concentration increased proportionally with the logarithm of the duration of infusion and corresponding to the infusion dosage. If it is assumed that the sulfonylureas affect the blood sugar indirectly via stimulation of insulin secretion, it may be concluded from these experiments that this stimulation is maximal with a certain moderate dosage and that larger doses given over prolonged periods of time exhaust the functional capacity of the islet cells. If this concept is correct, glucose tolerance tests immediately following the four-hour sulfonylurea infusion should show a delayed (diabetic) type of blood sugar curve. Instead, curves identical with those of untreated control animals were obtained, differing only in the level of the initial blood sugar level which was lower in the preinfused animals. The authors conclude from these experiments that the sulfonylureas do not exhaust the islet cells but cause a change in blood sugar homeostasis; "normoglycemia" is regulated to a lower level. This new level is maintained by hypersecretion of insulin, and the islet cell apparatus remains readily responsive to its normal stimuli, i.e., elevation of the blood sugar level. (German)

Haunz, E. A. (Grand Forks Clin., Grand Forks, N.D.): THE INSULIN REACTION: A CRITICAL REVIEW WITH ILLUSTRATIVE CASES. Journal-Lancet 77:183-90, June 1957.

A critical review of current concepts of the mechanism and treatment of insulin reactions is presented. The gruesome fact that insulin reactions may in exceptional cases cause irreversible brain damage or death should never be divulged to the diabetic patient, but this possibility should command the respect of every physician. So-called fatal cases of insulin reaction are often poorly documented and unconfirmed by autopsy. Actual proof is a genuine academic challenge. Insulin reactions may, in exceptional instances, bear an inverse ratio to the blood glucose level; that is, hypoglycemia may occur without reactions and vice versa. The physician should not use insulin therapeutically for any purpose unless he is equipped with the following:

a. A basic concept of the current theories of the mechanism of insulin reactions.

b. Ability to distinguish the signs and symptoms of reactions due to long-acting insulin from those due to crystalline insulin.

c. Keen awareness of the protean and sometimes bizarre nature of hypoglycemic signs and symptoms.

d. Competence in executing prompt and appropriate treatment for reactions of varied types and severity.

Hirsch, Edwin F. (Henry Baird Favill Lab. of St. Luke's Hosp., Chicago, Ill.): RENAL COMPLICATIONS WITH DIABETES MELLITUS (KIMMELSTIEL-WILSON'S DISEASE). Illinois M. J. 113:65-68, February 1958.

A descriptive report, well illustrated, of the necropsy findings in a diabetic expiring of renal failure. Papillary necrosis as well as nephrosclerosis was present.

Hirsh, B. (Manchester, England): STANDARDIZATION OF INSULIN. Brit. M. J. 1:827-28, April 5, 1958.

The author bemoans the present situation with regard to standardization of insulin in strengths of U20, U40 and U80, and lack of uniformity between unity markings of syringe and strength of insulin prescribed. He suggests that all insulin be standardized at 40 and 80 units per ml., and that all insulin syringes be calibrated at 40 U/ml. The adoption of these suggestions, while not wholly eliminating the possibility of confusion, would considerably diminish it.

Hollifield, Guy; and Parson, William (Dept. of Internal Medicine, University of Virginia Sch. of Medicine, Charlottesville, Va.): FOOD DRIVE AND SATIETY IN YELLOW MICE. Am. J. Physiol. 189:36-38, April 1957.

Spontaneous running activity during ad libitum feeding, fasting and refeeding was studied in inbred yellow mice. These studies suggest that the yellow gene per se is not associated with reduced activity and that inbred yellow mice have intact hypothalamic feeding centers. These findings suggest the obesity in yellow mice has a more complex etiology than reduction in activity and may be associated with an altered cellular metabolism especially in regard to fat.

Kaufman, Robert E. (Lenox Hill Hosp., New York, N.Y.): CO-EXISTENT LEUKEMIA AND DIABETES MELLITUS. Am. Pract.

& Digest Treat. 9:413-15, March 1958.

A case of co-existing leukemia and diabetes is reported. The diabetes became milder after radiation to the abdomen, possibly due to destruction of leukemic infiltrations in the pancreas or liver.

Knox, Lawrence J.; Harrison, Charles W.; and Doenges, John P. (Dept. of Medicine, Olney Sanitarium Clin., Olney, Ill.): THE USE OF AN ORAL HYPOGLYCEMIC AGENT AS AN ADJUVANT IN THE TREATMENT OF DIABETES MELLITUS. Illinois M. J. *112*:201-5, November 1957.

A clinical trial of Orinase in forty outpatient diabetics, deemed reasonably successful in thirty-five patients and a failure in severe and juvenile diabetics. A rise in serum cholesterol was observed in over half the patients.

Kulkoski, Bernard; and Duchateau, Norman (Green Bay, Wis.): OBSERVATIONS ON THE USE OF TOLBUTAMIDE IN GENERAL PRACTICE. Wisconsin M. J. 57:85-88, February 1958.

A group of twenty-one diabetics was studied in an attempt to find an adequate screening device for tolbutamide substitution for insulin on an ambulatory basis. It was demonstrated that by simple elimination of insulin, with blood sugar determinations on morning of substitution, and tolbutamide therapy for one week empirically, no untoward side effects were produced, even in those patients not responsive to tolbutamide therapy.

Lambert, Thomas H.; Bethard, William F.; Palmer, Solon, Jr.; and Monroe, Lee S. (Scripps Clin. and Res. Foundation, La Jolla, Calif.): ADMINISTRATION OF ORINASE (TOLBUTAMIDE): OBSERVATIONS OF EFFECT ON NONDIABETIC AND DIABETIC HUMANS. California Med. *88*:103-08, February 1958.

Of the thirty-seven diabetic patients taken at random who were treated with tolbutamide, control of diabetes was considered adequate in sixteen (43.5 per cent). This is a somewhat lower proportion than the 81 per cent and 73 per cent reported by other investigators, but it compares relatively well with the 48 per cent reported by Camerini-Davalos, Marble and Root in a series of cases selected to include only diabetes of the adult or stable type.

Laskowski, M., Jr.; Haessler, H. A.; Miech, R. P.; Peanasky, R. J.; and Laskowski, M. (Dept. of Biochemistry, Marquette University Sch. of Medicine, Milwaukee, Wis.): EFFECT OF TRYPSIN INHIBITOR ON PASSAGE OF INSULIN ACROSS THE INTESTINAL BARRIER. Science *127*:1115-16, May 9, 1958.

In experiments upon rats the authors demonstrated that a trypsin inhibitor of pancreatic origin facilitated the passage of commercial regular zinc insulin across the intestinal barrier as revealed by a significant fall in blood sugar after intraintestinal administration of insulin and inhibitor. Inclusion of the pancreatic inhibitor in tests upon the rate of insulin destruction in vitro disclosed that the inhibitor resulted in a reduced rate of insulin destruction.

Lobeck, Charles C.; and Forbes, Gilbert B. (Dept. of Pediat., University of Rochester Sch. of Medicine and Dentistry, Rochester, N.Y.): RESPONSE OF BONE TO ALLOXAN-DIABETIC ACIDOSIS IN THE RAT. Metabolism 7:133-40, March 1958.

Observations were made of changes in muscle and bone composition during alloxan-diabetic acidosis in rats. These revealed a decrease of 5.5 per cent in the crystal sodium content of bone and 8.3 per cent in the carbonate content. The sodium loss was shown to have originated in the rapidly exchangeable fraction of the crystal sodium. A decrease of 12 per cent in skeletal muscle potassium content and of 18 per cent in water content, with no change in sodium content, was also observed.

Luft, R.; Ikkos, D.; Gemzell, C. A.; and Olivecrona, H. (Dept. Endocrinol., Serafimerlasarettet; Dept. Obst. & Gynec., Karolinska sjukhuset; and Dept. Neurosurg., Serafimerlasarettet, Stockholm, Sweden): EFFECT OF HUMAN GROWTH HORMONE IN HYPOPHYSECTOMIZED DIABETIC SUBJECTS. Lancet *1*:721-22, April 5, 1958.

The authors report on the administration of growth hormone purified from human pituitaries to three hypophysectomized diabetic patients. In all three the hormone administration was followed by a pronounced increase in blood sugar level, glycosuria and acetonuria. In two of the patients the administration had to be stopped after thirty-six hours.

Lukens, F. D. W. (George S. Cox Res. Inst., University of Pennsylvania, Philadelphia, Pa.): CURRENT CONCEPTS OF DIABETES. Cincinnati J. Med. *38*:325-34, September 1957.

It is possible to formulate the simple objectives for the physician's attack on diabetes. These goals include the earlier diagnosis of diabetes by using our knowledge of the precipitating conditions in general practice and by the development of new methods of testing for this disease. A proper understanding of the permanence of the familial tendency and of the fluctuations of diabetes under treatment or intercurrent disease is essential to wise prognosis and good care. It is imperative to recognize differences in the severity of diabetes in different patients and in the same patient at different times. The principle objective of treatment is adequate nutrition. As applied to diabetes this means the utilization of enough carbohydrate to maintain each patient in safety and health. Until more is known, this should also be done to postpone the late complications of diabetes. The dietary, clinical and laboratory criteria for adequate nutrition as here defined have not been detailed. If the elementary facts about the disease are kept in mind, diet, insulin and laboratory tests can be used with good effect as shown already by the rising longevity in diabetes. The will to use these simple tools with utmost skill remains the first requirement for all interested in the management of diabetes.

Lundbæk, Knud; and Nielsen, Kai (Second University Clinic of Internal Medicine, and the Dept. of Pathology, Kommunehospitalet, Aarhus, Denmark): A COMPARATIVE STUDY OF THE ACTION OF THREE HYPOGLYCEMIC COMPOUNDS ON THE BLOOD SUGAR AND THE ISLET CELL OF THE PANCREAS IN THE RAT. Acta endocrinol. 27:325-38, March 1958.

A difference was found in the effect on the blood sugar of three hypoglycemic compounds. Both carbutamide and tolbutamide produced smooth blood sugar curves with a sustained hypoglycemic level, but the effect of tolbutamide was of shorter duration than that of carbutamide. This result is in accordance with the clinical findings in man.

With IPTD it was not possible to obtain such regular blood sugar curves. In doses between 0.5 and 0.7 gm. per kg. a diphasic blood sugar curve was always found. After 1.0 gm./kg.

profound hypoglycemia occurred, but most of the animals died a few hours after the injection. The hypoglycemic action of IPTD seems, therefore, to differ in some way from that of either carbutamide or tolbutamide. The secondary rise in the blood sugar seen after even small doses of IPTD may be the expression of a direct glycogenolytic effect of the compound on the liver, or it may be because it can evoke a counterregulatory response in the blood sugar regulation even in doses which do not produce severe hypoglycemia.

Lundbæk, Knud; Nielsen, Kai; and Rafaelsen, Ole J. (Second Clin. Int. Med. & Dept. Path., Kommunehospitalet, Aarhus Univ. Sch. Med., Aarhus, Denmark): MODE OF ACTION OF ORAL ANTIDIABETIC COMPOUNDS. Lancet *1*:1036-39, May 17, 1958.

The authors state that the present evidence of hypoglycemic effect of the oral hypoglycemic drugs cannot be explained by interference with the endocrine balance of the organism. Among the modes of action of these compounds inhibition of the output of glucose by the liver and enhancement of the uptake of glucose at the periphery seem to be acceptable. The details of the changes in enzyme activities probably involved in the hepatic and peripheral effects have not been elucidated. It is suggested that the hypoglycemic effect of the oral antidiabetic compounds is determined not by the presence or absence of insulin but by the degree of diabetic aberration of metabolism.

McCandless, Esther L.; and Zilversmit, D. B. (Dept. of Physiol., University of Tennessee, Memphis, Tenn.): FATE OF TRIGLYCERIDES AND PHOSPHOLIPIDS OF LYMPH AND ARTIFICIAL FAT EMULSIONS: DISAPPEARANCE FROM THE CIRCULATION. Am. J. Physiol. *193*:294-300, May 1958.

In studying the association of triglyceride, phospholipid, and cholesterol in plasma chylomicra during normal fat absorption, artificial fat emulsions labeled in either triglyceride or phospholipid component were administered to unanesthetized normal dogs to determine the disappearance rates of the two lipids from the circulation. Similar experiments were carried out using lymph from donor dogs fed I^{131}-triolein or P^{32}-phosphate with unlabeled triglyceride. Triglycerides were found to be rapidly removed from the circulation whether they were administered as an artificial fat emulsion or as a physiological emulsion, lymph. On the other hand, the emulsifying agents, liver lecithin or lymph phospholipid, required several hours for removal from the circulation. Evidence is presented that the slow disappearance rate of chylomicron phospholipids cannot be accounted for by exchange with plasma lipoproteins and indicates a slow removal of chylomicra phospholipid from the circulating blood. These findings suggest that in the transport of fat the phospholipids function repeatedly in forming an emulsifying surface layer around triglyceride particles reaching the circulation by absorption from the gastrointestinal tract or by mobilization from body fat depots.

McDonald, George (Lenox Hill Hosp., New York, N.Y.): DIABETES MELLITUS AND ADRENALECTOMY. A.M.A. Arch. Ophth. *59*:308, February 1958.

Diabetes has become one of the frequent causes of blindness today because the addition of insulin to our armamentarium enables an increasing number of diabetics to survive only to develop vascular complications. The incidence of diabetic retinopathy increases markedly with the duration of the disease, introducing a poor visual prognosis. It is theorized that overactivity of the adrenals due to marked changes in the blood sugar levels causes the production of the arteriolar lesions. Twenty cases of total bilateral adrenalectomy are reviewed, with the conclusion drawn that this procedure may be helpful if performed prior to the development of irreversible trophic changes.

Madison, Leonard L.; and Unger, Roger H. (Dept. of Internal Med., Univ. of Texas Southwestern Med. Sch., Dallas, and Dept. of Med., Veterans Administration Hosp., Dallas, Tex.): COMPARISON OF THE EFFECTS OF INSULIN AND ORINASE (TOLBUTAMIDE) ON PERIPHERAL GLUCOSE UTILIZATION IN THE DOG. Metabolism *7*:227-38, May 1958.

The comparison of the effects of insulin and tolbutamide on peripheral glucose utilization in the dog was studied. On a balanced diet and under standard testing conditions, a marked similarity in the effects of insulin and tolbutamide on peripheral glucose utilization is noted. The data presented indicate that the administration of tolbutamide to the intact dog duplicates the effects of insulin on peripheral glucose utilization. No effect on blood glucose concentration or blood glucose utilization was seen in the absence of beta cells of the pancreas, which lends further support to the thesis that the major physiologic action of tolbutamide is the release of stored insulin.

Mayer, Jean; and Vitale, Joseph J. (Dept. of Nutrition, Harvard Sch. of Public Health, Boston, Mass.): THERMOCHEMICAL EFFICIENCY OF GROWTH IN RATS. Am. J. Physiol. *189*:39-42, April 1957.

A simultaneous study of changes in food intake and body composition during the three months of postweaning growth was undertaken on three large groups of albino rats fed semisynthetic diets containing, respectively, 10, 25 and 60 per cent protein. The results show a remarkable similarity of percentage of body weight represented by protein during the whole growth period for the three groups and constancy within each group. The thermochemical efficiency (ratio of calories deposited to calories ingested) was constant from weaning to puberty and particularly so on the diet with the protein content most favorable for growth (25 per cent). The thermochemical efficiency was approximately constant from weaning to puberty on optimal diets and high protein diets, and declined somewhat earlier on a low protein diet.

Mills, Lewis C. (Sect. of Endocrinology, Dept. of Internal Medicine, Hahnemann Med. Coll., Philadelphia, Pa.): DIABETES IN THE AGED. Geriatrics *13*:320-26, May 1958.

Diabetes is a frequent problem in the aged population; one survey indicates that in 46 per cent of patients that develop diabetes the onset is after age fifty. While hyperglycemia and insulin requirements are on the average less than in the young, arteriosclerosis and its sequelae, ophthalmic problems, and diabetic neuropathy are often major causes of disability in the older diabetic. Treatment of the aged patient with diabetes is discussed.

Mosca, Leonardo (Dept. of Pathological Anatomy, University of Pavia, Italy): SOME EFFECTS OF TOLBUTAMIDE ON THE PANCREATIC ISLETS OF GROWING RATS. Quart. J. Exper. Physiol. *43*:265-69, July 1958.

Young white rats, when given 100 mg. tolbutamide per kilogram of body weight by mouth each day during the period of one and a half to four months of age, maintain the morphol-

ogy of the pancreatic islets at a juvenile stage. The alpha/beta cell ratio does not fall at puberty to the usual low value because of decreased beta-cell multiplication; yet the activity of these cells is generally increased (as revealed by their degranulation and enlargement). The persistence of many alpha cells is interpreted as compensatory to the hypoglycemia due to tolbutamide.

Olmsted, W. H.; and Oppenheimer, H. E. (St. Louis, Mo.): MASS SCREENING FOR GLYCOSURIA: COMPARISON OF COPPER REDUCTION (BENEDICT'S SOLUTION) AND GLUCOSE OXIDASE METHODS. Missouri Med. 55:358-60, April 1958.

The authors discuss mass screening methods for diabetes, based on previous experiences with Dreypak (Benedict's qualitative reagent) and compare this with recent experience in which this reagent was compared with Clinistix and Tes-Tape, both glucose oxidase methods. They conclude that the Benedict's test will detect 0.5 per cent as lowest level of sugar, whereas the oxidase methods detect urine sugar at 0.1 per cent level: Therefore, follow-up blood sugars will prove one out of five such people to be nondiabetic.

Proutt, T. E.; Weaver, J. A.; Scott, G. W.; and Asper, S. P., Jr. (Dept. of Med., Johns Hopkins Univ. Sch. of Med., Baltimore, Md.): EFFECT OF DIMERCAPROL (BAL) ON CARBOHYDRATE METABOLISM. Metabolism 7:240-55, May 1958.

Dimercaprol (BAL) has been previously shown to influence carbohydrate metabolism. The present study was undertaken to evaluate its effect in animals. The authors conclude that BAL is a mild hypoglycemic agent in the rabbit at a dosage of 0.18 mM/kg. They found it to be hyperglycemic when given a dosage of 0.36 mM/kg. and this, the latter effect, to be mediated through the adrenal medulla.

Riedel, Robert H.; and Pence, Virginia (Kansas State Board of Health, Topeka, Kans.): DIABETES MELLITUS: SCREENING BLOOD SUGAR TESTS IN KANSAS, 1956 AND 1957. J. Kansas M. Soc. 59:46-50, February 1958.

Approximately 11,000 diabetes screening blood sugar tests were performed by the State Board of Health in cooperation with the local medical profession in four experimental projects in three counties of Kansas in 1956 and 1957. Results suggested that it might be successfully continued on a broader scale depending upon good community organization, a full-time team of technical personnel, and adequate follow-up of positive cases.

Ritchie, Susan; and Waugh, Douglas (Dept. of Pathology, McGill University, Montreal, Quebec, Canada): THE PATHOLOGY OF ARMANNI-EBSTEIN DIABETIC NEPHROPATHY. Am. J. Path. 33:1035-57, November-December 1957.

The kidneys from five cases of Armanni-Ebstein diabetic nephropathy were studied by observation of conventional microscopic sections and by the technic of maceration and dissection of individual nephrons. The lesions in all cases consisted of marked glycogenic vacuolization of the epithelium of renal tubules in the outer medulla and innermost cortex. Tubules in the central and outer cortex were not affected. Dissection of nephrons showed the lesions to be localized to the terminal straight segment of the proximal convoluted tubule, with occasional extension into the contiguous portion of the thin limb. It was established in one case, and inferred in the others, that the exclusively cortical nephrons, which have a short Henle's loop and which do not penetrate the medulla, are relatively resistant to glycogenic vacuolization. The possible reasons for this reduced susceptibility to damage are considered.

Smellie, J. M. (Univ. of Birmingham, England): CARBUTAMIDE IN JUVENILE DIABETES. Brit. M. J. 1:553-55, March 8, 1958.

The author reports an inpatient study of three children whose diabetes was of very recent origin and who had little or no ketosis. The hypoglycemic action of carbutamide was demonstrated, and in two of the patients it was associated with a reduction in glycosuria. Such effects were achieved only with relatively high or potentially dangerous blood sulfonamide levels, and evidences of ill health of a nonspecific character, presumably due to sulfonamide intoxication, soon developed. These studies support the view of others that carbutamide has no place in the treatment of childhood diabetes, even if the disease is of recent origin and uncomplicated by ketosis. Failure of the patients to respond to the drug signifies that they do not still have effective endogenous insulin, and that assumption that the drug action is dependent on endogenous insulin remains unanswered.

Wright, Peter H. (Guy's Hosp. Med. Sch., London, England): PLASMA-INSULIN ESTIMATION BY THE RAT-DIAPHRAGM METHOD. Lancet 2:621-24, Sept. 28, 1957.

The author reports the determination of plasma-insulin activity of fifteen normal subjects by the rat-diaphragm method. Results confirmed previous suggestions that the effective insulin level is about 50 micro-units per ml. and is unlikely to be higher than 100 micro-units per ml. Nonketotic diabetics who did not require insulin therapy had similar fasting levels, whereas nondiabetic acromegalics had a fasting level about ten times as high as the normal. Administration of glucose to fasting subjects raised their plasma-insulin level in one hour. It was suggested that the dilution of plasma before assay released insulin by inactivating insulin antagonists or by releasing bound insulin, thereby causing apparently high results.

Zilversmit, D. B. (Div. of Physiol., University of Tennessee, Memphis, Tenn.): CURRENT CONCEPTS OF LIPIDE METABOLISM. Am. J. Med. 23:120-33, July 1957.

In considering the current concepts of atherogenesis, Dr. Zilversmit has undertaken the enormous task of summarizing and integrating the large and sometimes controversial recent literature on lipid metabolism. He begins with an account of the extraordinary advances in knowledge of the steps in biosynthesis of the lipids, notably, cholesterol, and in the intermediary metabolism of these compounds. He includes a broad discussion of the metabolic characteristics of lipid deposits, including atheromas, fatty liver and adipose tissue. The absorption and transport of lipids are next considered, with notable chapters on such aspects of clinical interest as the hyperlipemias and the role of clearing factor. Comprehensive and current, the experimental results thoroughly sifted by one expert in the field, this review is well worth a careful study for perspective in evaluating the special aspects of atherosclerosis which will be subjects of subsequent papers of this series.

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LAURANCE W. KINSELL, M.D., *Oakland*
RACHMIEL LEVINE, M.D., *Chicago*
KELLY M. WEST, M.D., *Oklahoma City*

PAST PRESIDENTS

HENRY T. RICKETTS, M.D., *Chicago;*
FREDERICK W. WILLIAMS, M.D., *New York;* JOHN A. REED, M.D., *Washington, D.C.*

Eighteenth Annual Meeting

The Eighteenth Annual Meeting of the American Diabetes Association was held in San Francisco June 21-22, 1958. Names of Officers and Councilors elected for the 1958-59 organizational year were reported in the July-August issue of DIABETES and are listed on the above masthead.

By all accounts, the recent meeting was one of the most rewarding ever held. Following are the registration figures, along with a comparison with previous years.

1958	Association Members	Guest Physicians	Other Guests	Total
San Francisco	267	165	27	459
1957				
New York	493	182	43	718
1956				
Chicago	406	130	29	565
1955				
Atlantic City	408	137	35	580
1954				
San Francisco	199	99	16	314
1953				
New York	438	198	7	643
1952				
Chicago	325	102	5	432
1951				
Atlantic City	342	125	10	477
1950				
San Francisco	162	83	6	251

SCIENTIFIC SESSIONS

Twenty-two scientific papers and the Banting Memorial Lecture were presented during the Scientific Sessions, and twenty-five were read by title. The Committee on Scientific Programs, under the Chairmanship of Francis D. W. Lukens, M.D., included the following members: John E. Howard, M.D., Vice Chairman; Alexander Marble, M.D., Vice Chairman; Thaddeus S. Danowski, M.D.; Harvey C. Knowles, Jr., M.D.; Henry E. Marks, M.D.; William C. Stadie, M.D.; L. O. Underdahl, M.D.; and Robert H. Williams, M.D.

The Banting Memorial Lecture was delivered by Jerome W. Conn, M.D., Professor of Medicine and Director of the Metabolism and Research Unit, University of Michigan Medical School; Chief, Department of Endocrinology and Metabolism, University Hospital, Ann Arbor, Michigan. Titled "The Prediabetic State in Man: Definition, Interpretation and Implications," it is published as the first article in this issue.

On invitation, James B. Field, M.D., winner of the 1958 Lilly Award, presented a paper entitled "Observations Concerning a Humoral Insulin Antagonist during Diabetic Ketosis."

ANNUAL BANQUET

The Banquet was held in the Peacock Court of the Hotel Mark Hopkins on Saturday evening, June 21, with 177 persons in attendance. After an invocation by Deaconess Maude Behrman, Director, the Mercer Memorial House, Atlantic City, New Jersey, John A. Reed, M.D., delivered the annual Address of the President.

ADDRESS OF THE PRESIDENT

JOHN A. REED, M.D., WASHINGTON, D.C.

Guests and members of the American Diabetes Association: The Address of the President certainly affords an open sesame of opportunities. One can review the accomplishments of historical events and great personages of vast vision and leadership who have preceded the present incumbent. Surely to do this is good, proper and acceptable. Then one has the opportunity to comment on the immediate activities of your organization during the preceding year. In this area one must extend words of appreciation to all who have taken part in them. This I do with profound sincerity and deepest thanks and say that without the devotion of many, the American Diabetes Association would be sterile of productivity. Again, this could be a chance to expound the problems of the present and offer "profound" solutions. I hesitate to do this because after many serious and prolonged deliberations of some of our present problems I would have disturbing dreams. I would see three opposing camps of thought: one crying that we are devotees of capitalism; a second loudly proclaiming we are servants of statism; and still a third fearing and opposing lay representation in our ruling bodies. But always the scene would change and all would be rallied around a banner bearing the inscription, "We stand for all that is best for the diabetic."

Surely this could be the occasion to project one's thinking into the future and even offer advice and admonition as to a direction to follow in the days and years to come. The latter procedure has at least two defects: One, crystal gazing is not scientific; and two, even if the pronouncements had an electric effect for the moment, the colorless photography of the subsequent printed record might result in a feeling of a cold gray dawn at a later date. I am sure, however, one could be on reasonably solid ground as to future reflections and prognostications in saying the pattern of the past, both as to basic principles and objectives and the selection of wise and broad-thinking leaders, esteemed advising Councilors, and dedicated committee workers, serves to assure a continued bright existence.

Let me venture to leave but one thought with you. This is neither new nor original with me but so profound it bears repetition. It is a direct quote from the Address of the first President of this organization. It is the first sentence of that address. To me it is an American Diabetes Association "first."

JOHN A. REED, M.D., PRESIDENT, 1957-58

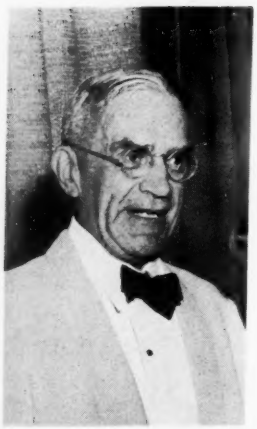
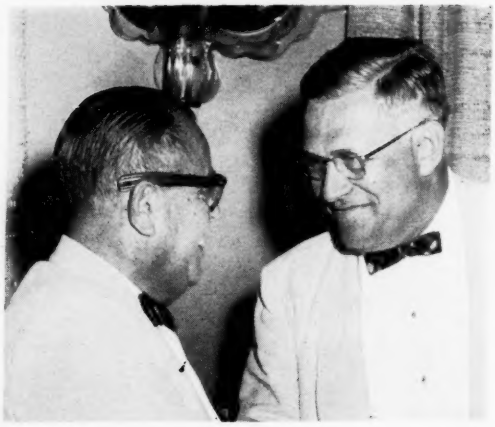
I quote Dr. Cecil Striker—our first President. "The patient suffering from the syndrome of diabetes mellitus is the reason for the existence of this Association." Dr. Charles Best essentially reiterated with changed wordage the same thought in his Presidential Address: "I regard the American Diabetes Association as an organization which is exploring the pathways which lead to the better treatment of diabetics."

In closing I simply add my own thought: May the gods of mercy bless this basic undertaking and give strength to all future leaders for continued fulfillment of this our primary purpose.

THE LILLY AWARD

Following the Address of the President, the 1958 Lilly Award consisting of $1,000 and a medal, the second to be made annually by Eli Lilly and Company for "independence of thought and originality in research in the field of diabetes," was presented to James B. Field, M.D., National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland. Dr. Field, who is pictured at the right, came from England, where he had been engaged in postdoctoral studies, to accept the award.

Dr. Field, thirty-two years of age, has studied the antagonist to insulin which he discovered in the serum of patients in diabetic acidosis; initiated a systematic investigation of the tolerance toward D vitamins as influenced by diabetes; been associated with research on the effects of sulfonamides upon diabetes; and studied a new technic of starch block electrophoresis for the separation of insulin from serum and for the detection of variations in the electrophoretic behavior of insulin in normal and abnormal sera.


At Banting Medal Ceremonies: WILLIAM H. OLMSTED, M.D. (left), JOHN A. REED, M.D. (shown with FREDERICK W. WILLIAMS, M.D., immediate Past President at right), and JEROME W. CONN, M.D., the Banting Memorial Lecturer.

THE BANTING MEDALS

During traditionally impressive ceremonies, Dr. Reed presented the Banting Medal to Jerome W. Conn, M.D., the Banting Memorial Lecturer. Dr. Reed, retiring President, was then awarded a Banting Medal by Frederick W. Williams, M.D., immediate Past President of the American Diabetes Association, "for distinguished service in the interest of doctor and patient."

In an unprecedented gesture, the Banting Medal also was awarded to William H. Olmsted, M.D., of St. Louis, upon his retirement as Treasurer of the Association, in appreciation for his six years of service in that position. Dr. Olmsted is Associate Professor of Clinical Medicine (Emeritus) at Washington University School of Medicine.

Guest speaker of the evening was William H. Thompson, Jr., of the Union Oil Company of California, whose address was "Let's Talk of Juvenile Decency." Mr. Thompson is a member of the Attorney General of California Citizens Advisory Committee on Crime Prevention.

ANNUAL BUSINESS MEETING

The Annual Business Meeting of the American Diabetes Association was held June 22. The statement by John A. Reed, M.D., President, was followed by: reports of the Secretary, Treasurer, Executive Director, and the Nominating Committee; and the Installation of the Incoming President.

Remarks by the President

I have no major statement to make except to say I have thoroughly enjoyed acting as your President under the edicts of many committees and the splendid administrative corps in the New York office. I wish to take this opportunity to thank all who have contributed, and as I said last night, without the help and assistance of all of these wonderful people your Association would be sterile as far as productivity is concerned.

In conclusion, I'd like to read a telegram that is addressed to the Association through me as President. "Greetings and good wishes to the American Diabetes Association from your fellow member agencies in the National Health Council on the occasion of your Eighteenth Annual Meeting. May your Sessions be rewarding as you review past progress and highlight continued advances in the control and treatment of diabetes." Norvin C. Kiefer, M.D., President, National Health Council.

JOHN A. REED, M.D.

Report of the Secretary

Mr. President, and members of the Association: It is always a matter of some difficulty to me to decide what to report as Secretary. The Secretary's job is sort of general factotum around this organization and I have tried to pick out a few items which I think will have interest.

The next Annual Meeting, the Nineteenth, will be held a year hence in Atlantic City prior to the AMA Meeting and again at Chalfonte-Haddon Hall.

The Seventh Postgraduate Course will be held in St. Louis, Jan. 21-23, 1959. The program practically has been finalized and we all feel that it is outstanding. We have felt that about each one, and so far each has surpassed the last.

The full membership of our organization is now 2,497, with twenty new applications pending. This is a net gain for the year of 166. The activities of the organization as a whole are continuing to grow, with more than 200 members actively participating in thirty-six groups and committees.

The journal DIABETES now has a circulation of more than 3,600 and of these more than 1,000 are paid subscriptions of nonmembers.

FORECAST circulation is over 54,000 paid subscriptions.

All of these activities have forced some further expansion of the New York central office and a new department has been set up primarily for subscription lists and mailing. A new activity of the Committee on Professional Education this year

was to conduct a national conference on teaching and research in diabetes. This was sponsored by the American Diabetes Association in Atlantic City on May 3 with the support of the National Institute of Arthritis and Metabolic Diseases. Representatives of a majority of American medical schools attended and it is believed that this conference will aid materially in improving present methods of teaching.

For more than a year our Subcommittee on Survey of Diabetes Abstracts Coverage, headed by Dr. Arnold Lazarow as Chairman, has been investigating ways and means of improving abstracts, particularly abstracts useful in the research area. A plan has evolved which seems to meet the need of research workers in diabetes. This plan already has been under discussion with the NIAMD and appears to provide another area where a joint program will facilitate research. It is hoped that this plan can be finalized and placed in operation some time next year.

In behalf of all of us I wish to extend again our many thanks and deep appreciation to Mr. Connelly and his devoted staff in the New York office, as it is only by their constant efforts and dedication to this work that it is made possible.

FRANKLIN B. PECK, SR., M.D.

Report of the Treasurer

This is the Treasurer's Report for 1957-58, or the Fiscal Year ending March 31, 1958.

INCOME: Income for 1957-58 was $347,944. In 1955-56 income was approximately $250,000. Our gross income is based mainly upon the fact that circulation of our publications is rapidly expanding and they have been so successful.

EXPENSE: The expenses this year of our Association were $343,066. The income exceeded the expense by $4,878, so we are in the black.

The annual earned income of the Association was $222,851 or 64 per cent of the total income. This money was earned through our publications and other miscellaneous sources. In other words two thirds of our total income is from self-supporting projects.

Out of every dollar of income, the percentage spent on our programs was as follows:

Professional Education	31.6
Patient Education	32.9
Public Education and Case Finding	20.2
Research	5.6
General Administration, Capital Expenditures and Depreciation	9.7

GIFTS: The Treasurer gratefully acknowledges the following gifts:

From Affiliate Associations

Diabetes Council of Public Health Federation, Cincinnati, $450
Diabetes Association of Greater Cleveland, $1,500
Fresno County Diabetes Association, $250
Lay Society of Greater Kansas City, $1,000
Los Angeles Diabetes Association, $200
Louisiana Diabetes Association, $16
Philadelphia Metabolic Association, $100
Lay Society of Reading Diabetes Association, $100
Washington Diabetes Association, $250
Nebraska Diabetes Association, $100
St. Louis Diabetes Association, $2,000

From Corporations

Ames Company, $20,000
A & S Supply Corporation, $75
Eli Lilly and Company, $50,000
Nehi Corporation, $100
E. R. Squibb & Sons, $10,000
Union Central Life Insurance Company, $500

From Foundations which gave $1,000 or more

Frueauff Foundation, $1,000
Paul B. Litchfield Foundation, $1,000
Rockefeller Brothers Foundation, $2,500
Stranahan Foundation, $1,000

From Bequests

Mary Jennings Lee Estate, $1,000

From Royalties

Book by Dale Evans Rogers, $2,430

From Individuals

Dr. Edwin W. Gates, $1,000
Wm. A. and Veronica Johnson, $3,000

WILLIAM H. OLMSTED, M.D.

Report of the Executive Director

Dr. Reed, Dr. Peck, Dr. Olmsted and members of the American Diabetes Association: It is a great pleasure to have the privilege of seeing all of you at this business session of the Eighteenth Annual Meeting. The very nature of a national organization makes it impossible for me personally to be acquainted with all of the members, a fact which I believe is underscored by the Secretary's statement that there are now nearly 2,500 members of our organization.

There is not sufficient time to submit a complete report. Dr. Peck covered many of the projects and activities of this past year, however I thought I might mention a few additional ones.

The Committee on Public Education and Detection plans to establish four pilot studies among the Affiliate Associations using glucose oxidase for processing Dreypaks. The deadline for applications is August 11. Also for the Affiliates, a speakers' bureau has been planned and details will be announced some time this summer.

There are four or five publications under way, most of which will be out in late summer or fall.

The results of the survey among large industries, along with the statement on employment as prepared by the Committee on Employment under the Chairmanship of Dr. Joseph T. Beardwood, Jr., will be issued as a pamphlet for plant management and personnel directors. An insert on tolbutamide for the DIABETES GUIDE BOOK FOR THE PHYSICIAN is scheduled, as well as a brochure describing our scientific exhibits.

I should also mention, although I think most of you know about it, that this fall we will publish an ADA FORECAST Recipe Book based on the recipes of Deaconess Maude Behrman which have appeared in the ADA FORECAST. It is anticipated this booklet will be 160 pages.

One of the most important publications planned, under the sponsorship of the Committee on Finance, is a brochure for members of the Association describing the functions and objectives of the organization.

Several articles have been planned for national magazines for release about the time of Diabetes Week this fall. They

are large magazines with substantial circulation which should have considerable impact.

Too, I'd like to remind members if I may that we have a new scientific exhibit entitled "Diabetes—Today and Tomorrow: The Expanding Role of the Doctor." The exhibit is being displayed for the first time at this meeting of the American Medical Association. I am sure you will all be pleased with it. The exhibit was planned and constructed under the guidance and direction of the Committee on Scientific Exhibits of which Dr. Marshall I. Hewitt is Chairman.

In closing I wish to say, as I did last year and several years before, that the American Diabetes Association is an organization of individual members and all members have the same rights and privileges. That is the principle upon which the national office functions. For myself and staff, we wish to extend to each and every one of you a cordial invitation to visit the national office. If possible it would be appreciated if you could write ahead of time, or give us a call, to make sure the person you want to see will be on hand to greet you. Meanwhile, for those of you who may not be in New York the coming year, I will be at the registration desk between three and five o'clock this afternoon and I'd be delighted to talk to each one of you. Thank you very much.

J. RICHARD CONNELLY

PRESIDENT REED: Thank you, Mr. Connelly, and I am sure that all of us are beyond words of expression of appreciation for the magnificent job that Dick does—for his appreciation of our problems and his vast knowledge of solutions thereof, and his cooperative spirit, not only with the Officers and Councilors but in his contacts with every member that he meets of our Association. I personally like to think that I had a wee bit to do with bringing Mr. Connelly to our Association. He has done a job beyond description.

Report of the Nominating Committee

The Nominating Committee recommends the following slate of Officers and Councilors:

For President, Dr. Alexander Marble, of Boston; for First Vice President, Dr. Francis D. W. Lukens, of Philadelphia; for Second Vice President, Dr. Franklin B. Peck, Sr., of Indianapolis; for Secretary, Dr. E. Paul Sheridan, of Denver; for Treasurer, Dr. Thomas P. Sharkey, of Dayton.

For Councilors for the term ending in 1961: Dr. Joseph T. Beardwood, Jr., of Philadelphia; Dr. Arthur R. Colwell, Sr., of Chicago; Dr. Joseph H. Crampton, of Seattle; Dr. Laurance W. Kinsell, of Oakland; Dr. Rachmiel Levine, of Chicago; Dr. Kelly M. West, of Oklahoma City.

To replace Dr. E. Paul Sheridan for the term on the Council expiring in 1959: Dr. Laurentius O. Underdahl, Rochester, Minnesota.

(*It was moved, seconded and voted that the nominations be closed. All nominees were duly elected.*)

This report, in the absence of the Committee Chairman, was given by Dr. Frederick W. Williams.

HENRY B. MULHOLLAND, M.D., *Chairman*
HENRY T. RICKETTS, M.D.
FREDERICK W. WILLIAMS, M.D.

Installation of Incoming President

JOHN A. REED, M.D., *retiring President*: It is a great pleasure indeed to present to you your next President, Dr. Alexander Marble, who I am sure needs no introduction, no biographical sketch or any other words concerning his previous standing, with this organization and with medicine in general.

As you all know, Dr. Marble has been associated with Harvard Medical School for many years; he has been identified with the Joslin Clinic in Boston for many years; and he is on the staff of the New England Deaconess Hospital in the same city. Dr. Marble is one of the greats in the field of diabetes. He has a discerning and understanding knowledge of diabetes, not only in the clinical phases but likewise in the research field. Members of the American Diabetes Association, it is indeed a great pleasure to pass the office of President and its gavel to Dr. Alexander Marble, your new President.

Remarks of Newly-installed President: Dr. Reed, members of the Association: I recognize and deeply appreciate the honor of election to the Presidency of the American Diabetes Association. You are so kind and trusting in making this selection that I will be inspired to do my very best to live up to the responsibilities of the office. These responsibilities are not inconsiderable in these days when physicians, teachers and medical investigators are affected greatly by changing world affairs, and the need for making decisions which may at times have far-reaching consequences of a medical and social nature.

I come to this office finding a tradition of unselfish service which has been established by Dr. Reed and other predecessors. With the help of the members of the Council, of the Board of State Governors, of the Committee members, of the membership at large, and of Mr. Connelly and his extraordinarily competent staff, I shall do my best not only to carry on the excellent work in progress but also to advance the cause of the Association in every way possible to the end that diabetes and persons with diabetes may benefit. Again, I thank you.

ALEXANDER MARBLE, M.D.

SEVENTH POSTGRADUATE COURSE

As previously announced, the Seventh Postgraduate Course in Diabetes and Basic Metabolic Problems will be held by the American Diabetes Association on Jan. 21, 22 and 23, 1959, in St. Louis, Missouri. William H. Olmsted, M.D., of St. Louis, will be Director, with Henry E. Oppenheimer, M.D., St. Louis, Co-Director.

The tentative program includes these major half-day subjects: "The Pathophysiology of Insulin," "The Treatment of Diabetes: General Principles," "Relationship of Lipid Metabolism to Diabetes," "Special Problems in the Management of Diabetes," "Complications of Diabetes," and "The Oral Hypoglycemic Agents."

Round-table conferences, a new feature in our Postgraduate Course series, will be held on January 22 at the close of the afternoon session. Registrants will be divided into groups of sixteen or less, so that questions may be addressed to members of the faculty who will include the following: Frank N. Allan, M.D.; Charles H. Best, M.D.; Arthur R. Colwell, Sr., M.D.; Jerome W. Conn, M.D.; T. S. Danowski, M.D.; Garfield G. Duncan, M.D.; Robert M. Kark, M.D.; Harvey C. Knowles, Jr., M.D.; Arnold Lazarow, M.D., Ph.D.; Rachmiel Levine, M.D.; Francis D. W. Lukens, M.D.; Alexander Marble, M.D.; Max Miller, M.D.; Henry T. Ricketts, M.D.; William C. Stadie, M.D.; and Frederick W. Williams, M.D.

The Course is open to Doctors of Medicine and was developed by the Association's Committee on Professional Education. It will be given in cooperation with the University of Missouri School of Medicine, St. Louis University School of Medicine and Washington University School of Medicine. Registration fees will be $50 for members of the Association and $100 for nonmembers. Send inquiries and applications to the national office of the American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

NINETEENTH ANNUAL MEETING

The Nineteenth Annual Meeting of the American Diabetes Association will be held in Atlantic City, New Jersey, June 6-7, 1959, prior to the annual session of the American Medical Association. Further information will be announced in future issues of DIABETES.

ADA AMENDS BYLAWS

The Council of the American Diabetes Association on June 20, 1958, adopted the following amendment of "Article X, Fiscal Year," of the Bylaws: "The fiscal year of the Association shall be such as the Council may from time to time determine."

WINNERS OF 1957-58 MEDICAL STUDENT-INTERN ESSAY CONTEST

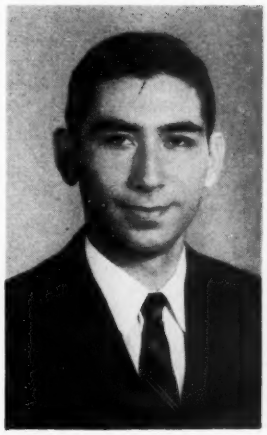
The Committee on Scientific Awards of the American Diabetes Association is pleased to announce the selection of Fredric Solomon, M.D., Chicago, and William Coleman Cohen, M.D., Baltimore, as winners of the 1957-58 (sixth) Medical Student-Intern Essay Contest.

Dr. Solomon won the award of $250, made possible by the St. Louis Diabetes Association, for the best paper in the field of diabetes reporting original work, whether laboratory investigation or clinical observation. Dr. Cohen received $50 for the best case report or review article.

Jessie E. Hano, New Orleans, and Frederick A. Spencer, Jr., Little Rock, both received honorable mention in the best case report or review article category, and one-year subscriptions to Volume 7 of DIABETES.

"Embryomegaly and Increased Fetal Mortality in Pregnant Rats with Mild Alloxan Diabetes" is the title of Dr. Solomon's prize-winning paper. Fredric Solomon was born in Chicago in 1936, and attended Chicago public schools and the University of Chicago Laboratory School, graduating at the age of fourteen, president of his class. The year previously, at the age of thirteen, he had appeared as a "Quiz Kid." Awarded a four-year, full-tuition John Crerar Scholarship to the College of the University of Chicago, he received a B.A. in 1954. He entered the University of Chicago School of Medicine at the age of eighteen and received his M.D. in 1958, when he was twenty-one. Dr. Solomon is at present serving a rotating internship at the University of Illinois Research and Educational Hospitals, Chicago. His interests lie in the fields of academic medicine (endocrinology) and psychiatry.

Dr. Cohen, whose paper is entitled "Severe Diabetic Neuropathy, A Case Study with Special Reference to the Autonomic Nervous System," was born in Baltimore in 1930. He attended Baltimore public schools, and was graduated from



FREDRIC SOLOMON, M.D. WILLIAM COLEMAN COHEN, M.D.

Baltimore City College in 1949. After receiving his B.A. from The Johns Hopkins University in 1952, he attended the University of Maryland School of Medicine where he received his M.D. in 1956. He served an internship and junior assistant residency at the University Hospital in Baltimore. He is now senior assistant resident in medicine at the University Hospital in Baltimore, and is interested in future training in endocrinology.

Mr. Hano, whose paper "Insulin Substitutes in Experimental and Clinical Diabetes," won honorable mention, was born in Covington, Louisiana, in 1935, and received his B.S. from Louisiana State University. At present he is a medical student and research-teaching assistant in the Department of Physiology, Louisiana State University School of Medicine.

Mr. Spencer, whose paper entitled "Insulin and Insulin Substitutes" also won honorable mention, was born in 1932, and educated in the public schools in Middletown, Connecticut. He received a B.A. in English from Salem College, Salem, West Virginia, in 1954, and an M.S. in Physiology from the University of Arkansas Medical School in 1957, his research and thesis dealing with hypoglycemic sulfonylureas. At present he is a student at the University of Arkansas School of Medicine, and laboratory assistant in the Departments of Physiology and Pharmacology.

THE 1958-59 MEDICAL STUDENT-INTERN ESSAY CONTEST

The 1958-59 (seventh) Medical Student-Intern Essay Contest is open to medical students, interns and physicians within two years after their graduation from medical school.

An award of $250 is offered to the author or authors of the best paper reporting original work, whether laboratory investigation or clinical observation. This prize again has been made possible by the St. Louis Diabetes Association. One hundred dollars ($100), double the award in previous years, will be given for the best review article or case report.

The papers will be judged on the basis of the value of the material and the method of presentation. Any subject relating to diabetes and basic metabolic problems may be selected.

Members of the American Diabetes Association and subscribers to DIABETES are asked to encourage medical students, interns and physicians within two years after their graduation from medical school to enter the contest. Those entering should submit the original and two copies of their manuscripts. Manuscripts should be typewritten, double-spaced, and mailed by April 1, 1959, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y.

1959 LILLY AWARD

As previously announced, the third annual Lilly Award will be given at the Nineteenth Annual Meeting of the American Diabetes Association, June 6-7, 1959, in Atlantic City, New Jersey. The following stipulations govern the contest for the award, which is supported by Eli Lilly and Company and consists of $1,000 and a medal.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before January 1 of the year of the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to January 1 of the same year of the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on the subject of his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and the medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

DEADLINE FOR RESEARCH FELLOWSHIP APPLICATIONS

The Committee on Research and Fellowships of the American Diabetes Association announces that applications for Research Fellowships must be received on or before Nov. 15, 1958. At least one Fellowship for the academic year 1959-60 will be awarded. Requests for application forms and inquiries should be addresed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

NOVEMBER 16-22 IS DIABETES WEEK

The forty-two Affiliate Associations of the American Diabetes Association and Committees on Detection of County and State Medical Societies are asked to note this date in making plans for development of Public Education and Detection programs. Members are urged to participate in their community campaigns.

The purpose of the year-round Diabetes Detection Drive, highlighted by Diabetes Week each year, is to find and help the nearly 1,000,000 men, women and children in this country who have diabetes but do not know it. The week which precedes Thanksgiving is the official Diabetes Week.

ADA FORECAST RECIPE BOOK

A book of recipes and menus for diabetics, written by Deaconess Maude Behrman, will soon be published by the American Diabetes Association. Indexed and styled for ready use, it contains hundreds of food specialties which will be enthusiastically received by diabetics, their families and friends. Additional information including the date of publication will be announced.

NEWS OF AFFILIATE ASSOCIATIONS

The CHICAGO DIABETES ASSOCIATION will conduct its second annual Symposium on Diabetes Mellitus at the Drake Hotel, Chicago, Monday, Nov. 17, 1958. The Woodyatt Memorial Lecture, entitled "The Islets of Langerhans," will be delivered by Francis D. W. Lukens, M.D., Professor of Medicine and Director of the George S. Cox Medical Research Institute, University of Pennsylvania School of Medicine. The following program also has been scheduled: "Action of Insulin," by Rachmiel Levine, M.D., Chairman of the Department of Medicine, Michael Reese Hospital, and Professorial Lecturer in Physiology, University of Chicago; "Aspects of Pathology of Diabetes Mellitus," by W. Stanley Hartroft, M.D., Ph.D., Mallinckrodt Professor and Chairman, Department of Pathology, Washington University, St. Louis; "Recent Concepts in the Early Recognition of Diabetes Mellitus," by Stefan S. Fajans, M.D., Associate Professor of Internal Medicine, Division of Endocrinology and Metabolism and the Metabolic Research Unit, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor; "Management of the Ambulatory Diabetic," by Arthur R. Colwell, Sr., M.D., Chairman, Department of Medicine, Northwestern University Medical School; "Management of Severe Diabetic Acidosis in Children," by Jack Metcoff, M.D., Chairman, Division of Pediatrics, Michael Reese Hospital, and Professor of Pediatrics, Northwestern University Medical School; "Panel Discussion of Oral Hypoglycemic Agents," Moderator: Henry T. Ricketts, M.D., Professor of Medicine, University of Chicago; "Problems of the Pregnant Diabetic," by Ralph A. Reis, M.D., Professor of

Obstetrics and Gynecology, Northwestern University Medical School; "Infants of Diabetic Mothers," by David Yi-Yung Hsia, Director of Research, Children's Memorial Hospital, Chicago, and Professor of Pediatrics, Northwestern University Medical School; "Insulin Antibodies," by Joseph Skom, M.D., Instructor in Medicine, Northwestern University Medical School, and Chief, Medical-Obstetrical Clinic, Northwestern University. Ralph E. Dolkart, M.D., is Chairman of the Committee on Scientific Programs.

Physicians registering for the course will be charged an enrollment fee of $25.00, with the exception of members of the Chicago Diabetes Association and the American Diabetes Association, who may enroll without cost. Inquiries should be addressed to Chicago Diabetes Association, 5 South Wabash Ave., Chicago 3, Ill.

The NEW JERSEY DIABETES ASSOCIATION in cooperation with the New Jersey State Department of Health will present its Sixth Annual Symposium on Diabetes Mellitus Oct. 29, 1958, at The Harrison S. Martland Medical Center, Newark, N.J. After visiting the exhibits in the morning, registrants will join ward rounds and bedside conferences from 11:00 a.m. till noon. Chief discussant will be Francis D. W. Lukens, M.D., Professor of Medicine and Director of the George S. Cox Medical Research Institute, University of Pennsylvania. Harold J. Jeghers, M.D., Professor of Medicine and Director of the Department of Medicine, Seton Hall College of Medicine, will be the moderator.

The afternoon session, a clinical conference, will begin at 2:00 p.m. and include these papers: "The Arteries and Kidneys in Diabetes Mellitus," by William E. Ehrich, M.D., Professor of Pathology, University of Pennsylvania Graduate School of Medicine and Chief of Pathology of Philadelphia General Hospital; "Renal Biopsy Pathology in Diabetic Patients," by George E. Schreiner, M.D., Associate Professor of Medicine and Director of the Renal Clinic, Georgetown School of Medicine; "Problems of Lipid Metabolism in Diabetes," by David Seligson, ScD., M.D., Associate Professor of Medicine, Yale University School of Medicine and Director of Laboratories, Grace-New Haven Community Hospital; "The Relationship Between Diabetes and Obliterative Arteriosclerosis," by Walter Redisch, M.D., Associate Professor of Clinical Medicine, New York University College of Medicine.

The last feature of the program will be a panel discussion entitled "Arteriosclerosis, Diabetes and Related Lipid Metabolism." Moderator will be Dr. Jeghers, and the panel members will be Drs. Lukens, Ehrich, Schreiner, Seligson and Redisch.

The NEW YORK DIABETES ASSOCIATION (Clinical Society) will present its Sixth Symposium Day on Diabetes Mellitus on Friday, October 10, at the Hunter College Playhouse Auditorium, 68th Street between Park and Lexington Avenues, New York. The Symposium is entitled "The Brain and Diabetes Mellitus." The following program has been arranged:

Morning: "Metabolism of the Brain and its Relation to Diabetes Mellitus," by Seymour S. Kety, M.D., Chief, Laboratory of Clinical Science, National Institute of Mental Health, and Professor of Physiology, Graduate School of Medicine, University of Pennsylvania; "The Pathological Effects of Hypoglycemia," by Harry M. Zimmerman, M.D., Chief, Laboratory Division, Montefiore Hospital and Professor of Pathology, College of Physicians and Surgeons, Columbia University; "The Hypothalamic Control of the Anterior Hypophysis and its Metabolic Implications," by Roger Guillemin, M.D., Associate Professor of Physiology, Baylor University College of Medicine; "The Role of the Highest Integrative Functions of the Central Nervous System in Disease," by Harold G. Wolff, M.D., Professor of Medicine (Neurology), New York Hospital-Cornell Medical Center.

Afternoon: "The Role of Environment and Personality in the Management of the Difficult Patient with Diabetes Mellitus," a panel discussion with Lawrence E. Hinkle, Jr., M.D., Associate Professor of Clinical Medicine, New York Hospital-Cornell Medical Center; Albert J. Stunkard, M.D., Associate Professor of Psychiatry, University of Pennsylvania Medical School; Alfred E. Fischer, M.D., Attending Pediatrician, Mt. Sinai Hospital; Harvey C. Knowles, Jr., M.D., Associate Professor of Medicine, University of Cincinnati College of Medicine. The afternoon session will conclude with "Clinical Experience with the Use of the Oral Hypoglycemic Agents," by Garfield G. Duncan, M.D., Professor of Medicine, University of Pennsylvania; "Surgery of Segmental Vascular Disease," by E. Stanley Crawford, M.D., Department of Surgery, Baylor University College of Medicine.

The names of individuals who will open the discussion on each paper will be announced later.

Physicians, medical students and other professional personnel interested in diabetes are cordially invited to attend. Admission will be by ticket only. While there is no fee, advance registration is required and may be made by calling or writing New York Diabetes Association, 104 East 40th St., New York 16.

The VIRGINIA DIABETES ASSOCIATION presented the following program on May 9 at the Eighth Annual Scientific Assembly of the Virginia Academy of General Practice May 8-11 at Virginia Beach: "Insulins in the Treatment of Diabetes," by Franklin B. Peck, Sr., M.D., Associate Professor of Medicine, Indiana University School of Medicine and Consultant in Medicine, Indianapolis General Hospital; "Current Problems in the Diagnosis and Management of Diabetes Mellitus," a panel discussion with Drs. Peck, Thomas S. Edwards, Charlottesville; William R. Jordan, Richmond; Jason McClellan, Newport News; and H. Clarkson Meredith, Jr., Norfolk.

NEWS NOTES

IDF ELECTS NEW OFFICERS

At the general Council Meeting on July 22-23 of the Third Congress of the International Diabetes Federation in Düsseldorf, Germany, the following officers were elected:

Honorary Presidents: Elliott P. Joslin, M.D., Boston; Charles H. Best, C.B.E., M.D., Toronto; B. A. Houssay, M.D., Buenos Aires; R. D. Lawrence, M.D., London.

Executive Board: President: Prof. Dr. J. P. Hoet, Louvain. Vice-Presidents: Ing. Mariano J. M. Ferraz, Sao Paulo; Mr. S. Lissauer, Amsterdam; Prof. Dr. K. Oberdisse, Düsseldorf; Prof. Dr. P. Rambert, Paris; Dr. Howard F. Root, Boston; Dr. M. Silvestri-Lapenna, Rome; Mr. V. Steenberg, Copenhagen; Madame G. Vernet, Geneva. Treasurer: D. H. Brown, A.C.A., London. Acting Secretary: Mr. James G. L. Jackson, London.

Dr. R. D. Lawrence served for six years as President and Professor Hoet, who succeeds him, served as Vice-President. Resignations were accepted with regret from Vice-President Monsieur Maurice Paz, Paris, and Ing. M. Cappetti, Montevideo, both of whom served as Vice-Presidents of the Federation since it was founded more than six years ago. Resignations were also received from Dr. and Mrs. L. L. Frank, London, Honorary Secretary and Executive Secretary respectively. Deep appreciation was expressed to them for capably filling these positions since IDF moved its headquarters to London after the Second Congress in 1955.

JACOBI FELLOWSHIP

The Women's Medical Association of the City of New York is offering the Mary Putnam Jacobi Fellowship, amounting to $2,000, to a graduate woman physician, American or foreign, to begin Oct. 1, 1959. Applications for the Fellowship, which is awarded for medical research, clinical investigation or postgraduate study in a special field of medicine, may be obtained by writing to Ada Chree Reid, M.D., Secretary, 118 Riverside Drive, New York 24, N.Y. Announcement of the winner of the award will be made no later than May 1, 1959.

AMERICAN COLLEGE OF PHYSICIANS ANNOUNCES FUTURE MEETINGS

The Annual Meeting of the American College of Physicians in 1959 will be held April 20-24 at the Conrad Hilton Hotel, Chicago. Eliot E. Foltz, M.D., Winnetka, Illinois, will be Chairman. The 1960 meeting of the College will be in San Francisco April 4-8. The headquarters are not yet selected. Chairman will be Robert F. Escamilla, M.D., San Francisco. In 1961 the meeting site will be the Americana Hotel in Bal Harbour, Florida; the dates May 8-12.

NATIONAL VITAMIN FOUNDATION FELLOWSHIP

The National Vitamin Foundation invites individuals holding doctorate degrees in medicine or one of the biological sciences, who are interested in continuing their training in the science of nutrition, to become candidates for a National Vitamin Foundation—Russell M. Wilder Fellowship. This postdoctoral Fellowship was created by the Foundation to honor Dr. Russell M. Wilder, former President of the American Diabetes Association, for his more than forty years of devoted service and significant contributions to medicine and public health and particularly for his leadership in advancing knowledge of diseases of metabolism and nutrition. The Fellowship is for three years and pays the recipient $4,500 the first year, $5,000 the second, and $5,500 the third year. It becomes effective July or September 1959.

QUANTITY PRICES FOR *FACTS ABOUT DIABETES*

FACTS ABOUT DIABETES is used by many physicians, clinic and hospital staffs to supplement their instructions. The thirty-two page booklet, published by the American Diabetes Association, offers invaluable information about diabetes both for the diabetic and the nondiabetic.

To aid those who wish to furnish booklets to classes and to new patients, the following prices have been established, offering a reduction in cost for purchase in quantity:

1-99 copies, $.25 each; 100-249 copies, $.20 each; 250-499 copies, $.195 each; 500-999 copies, $.1875 each; 1,000 copies and over, $.175 each. Order blanks are available upon request to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

VOLUME 7 BINDERS AVAILABLE

A binder for 1958 matching those for Volumes 1, 2, 3, 4, 5 and 6 (1952-57 issues) of DIABETES is available for immediate shipment at a price of $2.25. This insert binder is sturdy and attractive and will hold six issues of the magazine, plus the index supplement. Binders for Volumes 1, 2, 3, 4, 5 and 6 also are available at the same price. Address your orders to American Diabetes Association, Inc., 1 East 45th St., New York 17, N. Y.

PERSONALS

EDWARD L. BORTZ, M.D., of Philadelphia, will speak on "Planning for Good Health," at the National Conference on Individual Planning for Retirement sponsored by the Chamber of Commerce of the United States. The purpose of the conference, to be held on Oct. 2, 1958, at the Sheraton Hotel, Chicago, is to discuss the problems of life after retirement, including provision for adequate income, the establishment and preservation of a satisfactory home life, and the development of a program of useful activity.

JEROME W. CONN, M.D., Ann Arbor, and SAUL S. SAMUELS, M.D., New York, spoke at the Fourth Annual Scientific Meeting of the American College of Angiology, held in San Francisco on June 21-22. The title of Dr. Conn's paper was "Primary Aldosteronism and Hypertension." Dr. Samuels' paper was entitled "A Clinical Approach to Angina Cruris." In addition, Dr. Conn participated in a round-table discussion.

NECROLOGY

ACTIVE MEMBERS

Thomas F. Barrett, Lackawanna, New York, born July 22, 1905.

Frank B. Cross, Brooklyn, New York, born Oct. 20, 1879.

Richard N. DeNiord, Buffalo, New York, born Oct. 23, 1888.

Guillermo Garcia-Lopez, Vedado, Havana, Cuba, born Sept. 4, 1905.

James A. Henderson, Quincy, Illinois, born Sept. 27, 1907.

Robert W. Keeton, Chicago, Illinois, born July 7, 1883.

Louis A. Levison, Toledo, Ohio, born July 16, 1880.

Thomas McC. Mabon, Pittsburgh, Pennsylvania, born Aug. 11, 1890.

Richard C. Porter, Lancaster, New York, born Nov. 16, 1911.

Floyd A. Svec, Los Angeles, California, born April 11, 1917.

Nathan Swern, Trenton, New Jersey, born Jan. 28, 1897.

ASSOCIATE MEMBER

Walter B. MacGrath, Jr., Denville, New Jersey, born July 23, 1927.